The Journal of
Clinical Endocrinology and Metabolism

VOLUME 20 MARCH, 1960 NUMBER 3
Copyright 1960 by The Endocrine Society

A STUDY OF THE MECHANISM OF THE STEROID WITHDRAWAL SYNDROME. EVIDENCE FOR INTEGRITY OF THE HYPOTHALAMIC-PITUITARY-ADRENAL SYSTEM*

THOMAS T. AMATRUDA, JR., M.D., DOROTHY R. HOLLINGSWORTH, M.D., NICHOLAS D. D'ESOPO, M.D., G. VIRGINIA UPTON, B.A. AND PHILIP K. BONDY, M.D.

The Veterans Administration Hospital, West Haven, and the Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut

ABSTRACT

Cushing's syndrome developed in 10 male patients with pulmonary tuberculosis who were receiving prednisone, 30 mg. daily, and zinc-corticotropin (ACTH), 40 units every other day, for about six months in addition to antituberculosis chemotherapy. The responsiveness of the adrenal cortex to intravenous ACTH was tested before, during and after the period of prednisone-ACTH therapy. Although the baseline level of plasma cortisol before initiation of therapy was higher than that of normal controls, a smaller rise occurred in response to ACTH, resulting in similar final levels. During treatment both the baseline plasma cortisol level and the response to ACTH were reduced compared to normal controls. Despite very gradual tapering of the dosages of prednisone and ACTH after five to six months of therapy, striking symptoms developed, reminiscent of those occurring in Addison's disease or after bilateral adrenalectomy for Cushing's syndrome; these symptoms responded to reinsti-

Received August 1, 1959.

* Presented in part at the Eastern Sectional Meeting (December 6, 1957) and National Meeting (May 4, 1958) of the American Federation for Clinical Research (abstracts: *Clin. Res.* 6: 26, 253, 1958).

Aided by Grant #A 254 (C5) from the Division of Grants, National Institutes of Health, U. S. Public Health Service.

tution of prednisone therapy. However, both the baseline plasma cortisol level and the response to ACTH were normal, even while the patients were experiencing withdrawal symptoms. By utilizing insulin-induced hypoglycemia, the integrity of the hypothalamic-pituitary-adrenal system was evaluated in both the steroid-ACTH withdrawal patients and in controls who also had pulmonary tuberculosis. Surprisingly, the steroid-ACTH withdrawal patients experienced fewer hypoglycemic symptoms following the administration of 0.15 unit of insulin per Kg. intravenously than did the controls, associated with a significantly smaller decline in blood sugar concentration. Furthermore, they exhibited normal hypoglycemia responsiveness and a normal rise in plasma cortisol concentration in response to the hypoglycemia. These data indicate that, under the conditions studied, the hypothalamic-pituitary-adrenal system was intact following withdrawal of steroid-ACTH therapy despite the development of withdrawal symptoms. The etiology and pathogenesis of the withdrawal symptoms in these patients remain to be elucidated.

INTRODUCTION

A SYNDROME characterized by fever, anorexia, nausea, lethargy, arthralgia, desquamation, weakness and weight loss has been reported to develop in patients with spontaneous hyperadrenocorticism ten days to several weeks after they have undergone adrenalectomy (1–3). Many of the symptoms suggest the presence of adrenocortical insufficiency and it has been hypothesized that the syndrome is due either to relative adrenal insufficiency in patients whose tissues are conditioned to high levels of adrenocortical steroids (1, 3) or to "acute nutritional deficiency due to nutritional repletion after correction of hyperadrenocorticism" (2). A somewhat similar syndrome has been described in some patients with rheumatic diseases during gradual withdrawal of steroid therapy (4).

The present report concerns a study of adrenocortical responsiveness and the functional status of the hypothalamic-pituitary-adrenal system in this syndrome when it occurred after withdrawal of steroid-corticotropin therapy in patients with pulmonary tuberculosis who had received prednisone and corticotropin (ACTH).

The patients had been treated with both prednisone and ACTH to try to prevent the development of adrenocortical atrophy, known to occur in experimental animals (5) and man (6) during the administration of adrenocortical steroids. Concurrent prednisone-ACTH therapy had been selected since a strong possibility existed that some of the patients might abruptly discontinue their therapy and leave the hospital.

The major objective of the study was to evaluate the effect of the hormones on the clinical course of pulmonary tuberculosis. However, it was hoped incidentally to assess the ability of ACTH to prevent adrenocortical atrophy and to gain objective information bearing on the widespread impression that chronic hyperadrenocorticism may result in chronic adreno-

cortical insufficiency long after the hyperadrenocorticism has been corrected.

The data obtained demonstrated that the hypothalamic-pituitary-adrenal system was intact despite the long period of steroid-ACTH therapy and the occurrence of symptoms after withdrawal of these hormones.

MATERIALS AND METHODS

Ten male patients with advanced pulmonary tuberculosis received prednisone, 10 mg. three times daily, and zinc-corticotropin, 40 units every other day, in addition to conventional antituberculosis chemotherapy, employing streptomycin, isoniazid and paraaminosalicylic acid in various regimens. The chemotherapy was started a few days before initiation of the prednisone-ACTH therapy, which was arbitrarily continued for five to six months. With that regimen, definite signs of Cushing's syndrome developed in all of the patients. After five or six months the prednisone dosage was slowly tapered over a period of three weeks and then discontinued. Zinc-ACTH was continued throughout the tapering period and for an additional four days. The functional status of the adrenal cortex before, during and after withdrawal of prednisone-ACTH therapy was tested by measuring the rise in plasma cortisol concentration in response to 25 units of ACTH infused over a five-hour period. Plasma cortisol was determined by the method of Bondy *et al.* (7), employing quantitative paper chromatography and photofluorometry.

The integrity of the hypothalamic-pituitary-adrenal system after withdrawal of steroid-ACTH therapy was investigated by measuring the response of blood sugar and plasma cortisol levels to the administration of insulin. After an overnight fast, both the steroid-ACTH withdrawal patients and control subjects who also had pulmonary tuberculosis received glucagon-free insulin intravenously through an indwelling Cournand needle. Originally, an insulin-glucose tolerance test was performed employing 0.1 unit of insulin per Kg. of body weight and administering 0.8 Gm. of glucose per Kg. orally half an hour later. This procedure was discarded since it did not uniformly cause a rise in the concentration of plasma cortisol in normal persons. Next, a standard insulin tolerance test employing 0.1 unit of insulin per Kg. was tried (8). However, the insulin dose was soon increased to 0.15 unit per Kg. when it became apparent that not all patients were experiencing the expected degree of hypoglycemia. Blood was withdrawn at 0, 30, 60, 90, 120 and 180 minutes after administration of insulin for determination of blood sugar by the Somogyi-Nelson method (9), and at 0, 60 and 120 minutes for determination of plasma cortisol. The patients were under continuous observation throughout the procedure. Blood pressure, pulse rate and other vital signs were checked at frequent intervals and glucose, hydrocortisone sodium succinate and norepinephrine were available for immediate intravenous use if necessary. Antituberculosis chemotherapy was temporarily discontinued twenty-four hours before the insulin tolerance test was performed.

Nine of the 10 patients were experiencing definite withdrawal symptoms of varying severity at the time of the ACTH test and 4 had withdrawal symptoms at the time of the insulin tolerance test. During the study 4 patients required temporary reinstitution of ACTH and/or steroid therapy for control of severe symptoms which developed during withdrawal. In these cases the second course of steroids was usually brief, with tapering of dosage and withdrawal within a few weeks. In some patients, in the early part of the study, only the ACTH test was performed during the first withdrawal period

and the insulin tolerance test was performed during the second withdrawal period. In patients studied later, both tests were performed during the same withdrawal period and the insulin tolerance test was always carried out first, followed in twenty-four or forty-eight hours by the ACTH test. At the time of the final ACTH or insulin tolerance tests, both the steroid-ACTH and the control patients had entered a period of relative clinical stability in regard to the activity of their pulmonary tuberculosis. However, a few of the steroid-ACTH patients showed slight but definite temporary increases in their abnormal radiographic findings immediately after steroid-ACTH withdrawal. The steroid-ACTH withdrawal patients and the "control" patients with pulmonary tuberculosis were similar with respect to age and body weight. However the "control" patients generally had less advanced pulmonary tuberculosis than those in the steroid-ACTH withdrawal group.

Intravenous glucose tolerance tests were performed in the controls and the steroid-withdrawal patients weeks to months following withdrawal. After obtaining blood specimens with the patient in the fasting state, 25 Gm. of glucose as 50 per cent dextrose in water was injected intravenously and blood was withdrawn at one and two hours for determination of blood sugar concentration.

RESULTS

In every patient prednisone-ACTH therapy produced definite Cushing's syndrome, usually characterized by a gain in weight, plethora, truncal obesity, and dorsal and supraclavicular fat pads. Acne and purple abdominal striae were only occasionally noted. The combined hormonal-chemotherapeutic regimen also had a striking beneficial effect upon the course of the pulmonary tuberculosis, as will be described in detail elsewhere.

The results (mean) of the ACTH tests performed before, during and after withdrawal of prednisone-ACTH therapy are recorded in Table 1, along with data obtained on 17 normal subjects.

The patients were both acutely and chronically ill before initiation of

TABLE 1. RESPONSE OF PLASMA CORTISOL LEVEL TO ACTH (25 UNITS/5 HRS. I.V.)

	No. of subjects	Plasma cortisol (μg./100 ml.—mean $\pm$ S.E.)		
		Baseline	5 hrs.	Rise
Pulmonary tuberculosis treated with prednisone-ACTH:				
Pre Rx	10	15.6 $\pm$ 2.3*	32.9 $\pm$ 3.7	17.3 $\pm$ 2.7*
During Rx	10	6.3 $\pm$ 1.2†	22.1 $\pm$ 3.2†	15.8 $\pm$ 2.5†
After withdrawal of Rx	9	10.3 $\pm$ 1.9	31.4 $\pm$ 2.8	21.1 $\pm$ 2.5
Normal controls	17	10.7 $\pm$ 0.9	36.2 $\pm$ 1.5	25.5 $\pm$ 1.5

* $P < .02$ compared to normal controls.

† $P < .01$ compared to normal controls.

therapy with drugs or hormones. At that time they exhibited a higher than normal baseline level of plasma cortisol, but a smaller rise in response to ACTH, so that the final plasma cortisol concentration was not significantly different from that found in the normal controls, and the secretory rates of cortisol were probably also comparable. It is known that the rate of removal of cortisol from the plasma is proportional to its concentration. Therefore, a better index of the secretory rate of the adrenal cortex is afforded by the final plasma concentration obtained in response to ACTH rather than by the degree of rise from the baseline level, assuming that neither the volume of distribution nor the removal rate of cortisol changes (10, 11). Within four to six weeks after initiation of combined chemohormonal therapy there was remarkable clinical improvement; the patients were afebrile, asymptomatic and gaining weight. Repetition of the ACTH test at that time revealed that the mean baseline level of plasma cortisol was significantly less than normal, with a diminished response to ACTH resulting in an abnormally low final level. For reasons previously cited, it may be inferred that the secretory rate of cortisol from the adrenal cortex was probably impaired. This suggests that the concurrent administration of ACTH had not completely prevented adrenocortical atrophy due to the administration of prednisone, although the adrenal obviously retained a significant capacity to secrete cortisol.

Despite the gradual tapering of the dosage over a three-week period and continuation of ACTH for four additional days, symptoms developed in all but one of the patients upon withdrawal of steroid-ACTH therapy. These varied in severity but usually were quite striking. They included anorexia, nausea, weight loss, fine desquamation (particularly involving the face, hands and feet), vague musculoskeletal aches, arthralgia, fever, weakness and lethargy. The symptoms were suggestive of acute adrenal insufficiency and, indeed, in the 4 patients in whom steroid therapy was reinstituted the symptoms promptly abated. However, ACTH tests performed at the time symptoms were present, surprisingly revealed a normal baseline level of plasma cortisol and a normal response to ACTH (Table 1), indicating normal adrenocortical responsiveness.

These results suggested that if the withdrawal symptoms were due to hypoadrenocorticism the hypofunction was not due to a suppressed adrenal cortex but rather to hypofunction of the hypothalamus or adenohypophysis. That possibility was investigated by performing insulin tolerance tests in 7 of the 10 patients. Insulin tolerance tests were not performed in 3 patients: in Patient R. Kit. (one of the first subjects studied) an insulin-glucose tolerance test was performed instead; the 2 others were excluded because of the presence of hepatitis due to pyrazinamide therapy and of diabetes mellitus respectively. All 7 patients studied had experienced with-

drawal symptoms after discontinuation of therapy and in 4 instances steroid therapy had to be reinstituted for symptomatic relief. Since the unexpected results of the ACTH tests were not known until after a number of patients had already completed their therapy, insulin tolerance tests were carried out at intervals varying from two days to six months after steroid-ACTH withdrawal. However, 4 of the patients were having definite withdrawal symptoms of varying severity at the time the insulin tolerance test was performed. Their response to the test was similar to that of the 3 who were not experiencing withdrawal symptoms. The insulin tolerance tests were carried out with some trepidation since it seemed possible that the steroid-ACTH withdrawal patients would be extremely sensitive to insulin. However, they had far fewer symptoms during the test than did the control subjects with pulmonary tuberculosis. Most of the steroid-withdrawal and control patients manifested lethargy during the test. Only 2 steroid-withdrawal patients became "lightheaded" compared to 6 of the control subjects, 4 of whom also demonstrated objective difficulty in cerebration as tested by ability to read, repeat numbers and perform simple arithmetic. These tests were readily performed by all steroid-withdrawal patients. Hunger and profuse perspiration were also much more common and more severe in the control subjects. Two of the steroid-withdrawal patients had no hypoglycemic symptoms, whereas all of the controls had some symptoms and one even became comatose.

The impression that the withdrawal patients were less sensitive to insulin than were the controls was substantiated by determination of the blood sugar level at various intervals after administration of insulin. The control and steroid-ACTH withdrawal groups were similar with respect to age, body weight, and dose of insulin administered. As shown in Figure 1, the concentration of blood sugar at 30 minutes was significantly higher in the withdrawal group than in the controls, with mean values $\pm$S.E. of 47.4 ± 5.4 and 30.7 ± 3.0 mg. per 100 ml., respectively. The level of blood sugar initially, and at 60, 90, 120 and 180 minutes after insulin, did not differ between the two groups, and had returned to the fasting baseline by 180 minutes after insulin. Therefore, the steroid-ACTH withdrawal patients exhibited a decreased sensitivity to insulin and normal hypoglycemia responsiveness, since they were able to restore the concentration of blood sugar to the initial fasting level.

When the plasma cortisol levels of the two groups were compared, no significant differences were found. Both groups had normal fasting concentrations of plasma cortisol and both exhibited a significant rise of similar degree, despite the fact that in the withdrawal patients the hypoglycemia was less than in the controls (Fig. 1).

Early in the study it became apparent that not only a moderately severe

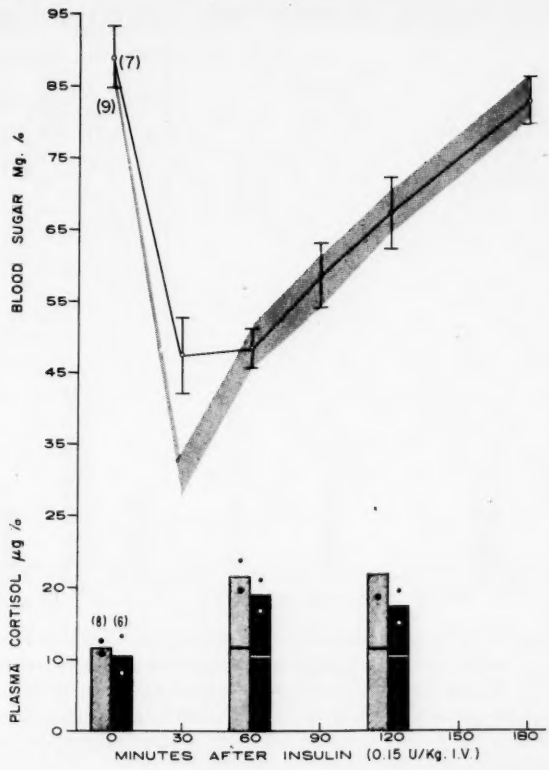


FIG. 1. *Insulin tolerance in steroid-ACTH withdrawal patients and control subjects.* The mean blood sugar level (mg./100 ml. ± S.E.) is indicated by the stippled area (controls) and by the black line (steroid-ACTH withdrawal group). The latter patients were significantly less sensitive to insulin than the controls since their blood sugar level at thirty minutes was significantly higher than that of the controls ($P < .02$). The blood sugar level did not differ significantly from that of the controls at other times. The mean levels of plasma cortisol are depicted by the heights of the gray (control) and black (steroid-ACTH withdrawal) columns; the dots above and below represent ± S.E. In both groups there was a significant rise in response to hypoglycemia. The groups did not differ significantly from one another.

but a prolonged hypoglycemia was necessary to insure a rise in the level of plasma cortisol, even when the hypothalamic-pituitary-adrenal system was intact. Figure 2 depicts data obtained on one of the first patients studied, employing an insulin-glucose tolerance test. The concentration of blood sugar fell only to 46 mg. per 100 ml. at 30 minutes and then rapidly rose to 83 mg. per 100 ml. at 60 minutes in response to oral glucose. The concentration of plasma cortisol did not rise. However, a normal rise was obtained

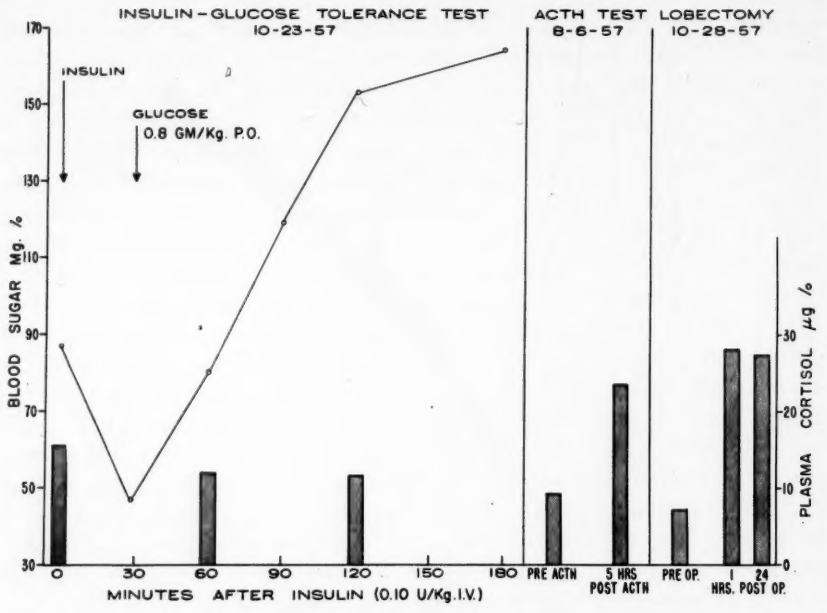


FIG. 2. *Patient R. Kit. (steroid-ACTH withdrawal)—Insulin tolerance and ACTH tests.* This patient had a virtually normal insulin-glucose tolerance curve, demonstrating hypoglycemia responsiveness. However, the level of plasma cortisol did not rise, although earlier it responded normally to ACTH, and later to pulmonary surgery. The hypoglycemia was of very brief duration and was asymptomatic since the patient received glucose at thirty minutes.

both in response to ACTH and in response to major thoracic surgery. Another withdrawal patient did not experience a rise in response to the insulin tolerance test, but again the blood sugar level declined to only 70 mg. per 100 ml. at 30 minutes and 59 mg. per 100 ml. at 60 minutes, indicating a definite decrease in the sensitivity to insulin. The importance of the duration of the hypoglycemia in producing a rise in plasma cortisol concentration was demonstrated in a control patient who experienced an abrupt fall in the blood sugar level to 20 mg. per 100 ml. at 30 minutes, rapidly followed by coma and twitching (Fig. 3). Glucose (25 Gm.) was promptly administered intravenously and consciousness was immediately restored. Although the brief period of hypoglycemia was very severe, no rise in the level of plasma cortisol occurred. The patient was carefully evaluated for any clinical or laboratory signs of hypopituitarism or primary hypoadrenalism, and no evidence for either was found. An ACTH test was performed and induced a normal rise in plasma cortisol concentration. The baseline levels present during both the insulin tolerance test and the ACTH

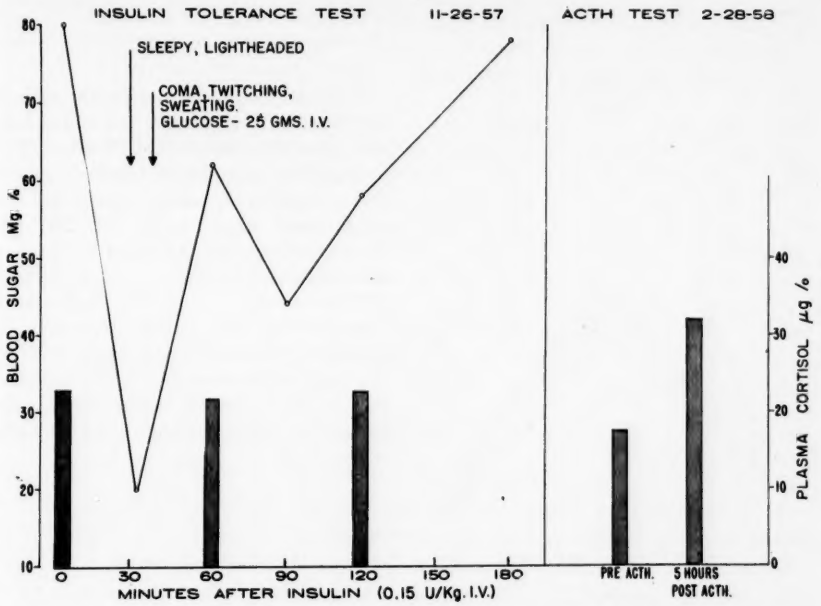


FIG. 3. *Patient R. K. (control)—Insulin tolerance and ACTH tests.* Failure of severe hypoglycemia of very brief duration to cause a rise in plasma cortisol concentration in a control subject. He later responded normally to a single infusion of ACTH and had no clinical or laboratory evidence of hypopituitarism. The high baseline cortisol level was probably due to moderate anxiety.

test were slightly elevated, probably reflecting the patient's tendency towards anxiety during almost any procedure.

During prednisone-ACTH therapy mild diabetes mellitus developed in 1 patient, necessitating treatment with insulin. For that reason, an insulin tolerance test was not performed after steroid-ACTH withdrawal. Since the withdrawal patients were less sensitive to insulin than were the control subjects, the possibility was considered that mild subclinical diabetes mellitus might have developed in additional patients as a result of the prednisone-ACTH therapy. Intravenous glucose tolerance tests were carried out in 9 withdrawal patients and in 9 of the controls with pulmonary tuberculosis (after an overnight fast) by administering 25 grams of glucose and determining blood sugar concentration initially and at 60 and 120 minutes after glucose. The results illustrated in Figure 4 show that the withdrawal patients could be separated into two groups, depending on whether or not the blood sugar level at 120 minutes had returned to the fasting baseline. In 5 patients the values fell within the range of those in the control group, but in 4 there was evidence of slight impairment of carbohydrate tolerance.

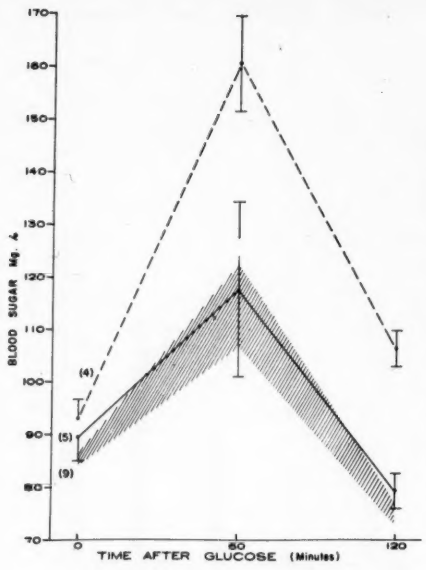


FIG. 4. *Glucose tolerance in steroid-ACTH withdrawal patients and control subjects.* After an overnight fast, 50 ml. of 50% dextrose was administered intravenously. The cross-hatched area represents the mean blood sugar level (mg./100 ml. ±S.E.) of 9 controls. The solid and broken lines represent the mean ±S.E. in steroid-ACTH withdrawal patients arbitrarily separated into two groups depending on whether or not the blood sugar level at two hours returned to the fasting baseline. Two of the 4 patients represented by the broken line had not eaten supper the night before the test.

However, 3 of these 4 patients complained of a chronically poor appetite, and 2 of them had not eaten any supper the night before the glucose tolerance test. The 5 patients, whose carbohydrate tolerance curves were normal, had also demonstrated decreased sensitivity to insulin during the insulin tolerance test. Therefore, it seems unlikely that the decreased sensitivity to insulin was due to the presence of subclinical diabetes.

It was hoped to assess the function of the hypothalamic-pituitary-adrenal system in other ways, such as measuring the response of plasma cortisol to major pulmonary surgery. However, only 1 of the patients treated with the prednisone-ACTH regimen has required surgery thus far. Blood was drawn for determination of plasma cortisol shortly before induction of anesthesia, and again about one hour after recovery from anesthesia. The patient (R. Kit.) tolerated right upper lobectomy very well without steroid therapy during the operation. Table 2 records the preoperative and postoperative plasma cortisol levels in Patient R. Kit. and in 5 control subjects undergoing comparable pulmonary surgery. Patient R. Kit. experienced a normal rise in plasma cortisol concentration in response to anesthesia and surgery. His relatively low preoperative plasma cortisol level is of interest; however, it fell within the normal range. He was extremely unconcerned about the approaching surgery and that attitude contrasted strongly with the normal apprehension of the control subjects. Control Patient S.P. recovered very slowly from anesthesia, and required mechanically aided ventilation for two hours postoperatively.

TABLE 2. EFFECT OF PULMONARY SURGERY ON PLASMA CORTISOL LEVEL

Subject	Status	Plasma cortisol (μg./100 ml.)	
		Preop.	Postop.
R. Kit.	S.W.*	7.3	28.1
H.D.	Control	19.8	35.9
S.P.	Control	19.0	62.7†
F.S.	Control	15.6	25.5
W.L.	Control	15.0	35.6
W.E.	Control	19.2	28.4

* Steroid-ACTH withdrawal patient.
† Slow recovery from anesthesia.

DISCUSSION

A primary objective of this study was to determine whether the adrenal atrophy produced by the chronic administration of adrenocortical hormones could be prevented by the simultaneous chronic administration of ACTH, as demonstrated in the rat by Ingle and Kendall (5). Although the occurrence of adrenocortical atrophy during steroid therapy has been unequivocally demonstrated, the status of the hypothalamic-pituitary-adrenal system after withdrawal of steroid therapy has not been firmly established. The literature contains numerous reports emphasizing the hazard that surgery or other stress may precipitate adrenal insufficiency in patients who have had adrenal steroid therapy sometime in the past. However, when these articles are critically reviewed, they almost invariably either record patients whose steroid therapy was abruptly discontinued just before or during the stress, or else base their conclusions on the tenuous grounds of "shock unresponsive to usual measures, responding to administration of adrenal steroids." The reports are too numerous to cite here, but they point up the need for comparable studies documented by objective measurements of adrenocortical function. One report, however, presents a plausible instance of adrenocortical atrophy verified at autopsy in a surgical patient who had not received any sustained systemic steroid therapy for four months (6). When adrenal steroids are suddenly withdrawn from patients who have been receiving them chronically, a period of anatomic (6) and functional (12, 13) adrenocortical insufficiency results. There is evidence of spontaneous recovery within five to twenty days (6, 12), and marked acceleration of recovery due to administration of ACTH (12, 13).

The present study differs from the foregoing reports in certain respects. Zinc-corticotropin was administered during the entire period of steroid therapy, and a striking degree of hyperadrenocorticism was maintained

throughout the study. Since the hormonal regimen was not varied, and the patients did not have a chronically active inflammatory disease such as lupus erythematosus or rheumatoid arthritis, the clinical picture of spontaneous hyperadrenocorticism was simulated closely. Of interest are the initially high baseline concentrations of plasma cortisol when the patients were acutely ill before antituberculosis therapy was instituted. Similar findings have been reported in patients with acute illnesses (14) and may be accompanied by a supernormal response to ACTH. However, our patients did not exhibit an excessive response to ACTH. The low baseline level of plasma cortisol and the subnormal response to ACTH during steroid-ACTH therapy suggested either that the concurrent ACTH therapy had not completely prevented atrophy of the adrenal cortex or that the plasma was being cleared of steroids at an accelerated rate. Nevertheless, the normal baseline level and normal response to ACTH found after withdrawal of steroid-ACTH therapy clearly indicate that physiologically significant adrenocortical atrophy was not present at that time.

While the study was in progress, Young and associates (15) reported the effectiveness of various ACTH regimens in maintaining adrenocortical responsiveness during steroid therapy in patients with rheumatoid arthritis, lupus and bronchial asthma. From measurements of urinary 17-hydroxycorticoids they concluded that a dosage of 100 units of corticotropin-gel injected twice weekly would prevent adrenal atrophy, whereas single weekly doses as high as 320 units were not consistently effective.

The development of symptoms suggestive of adrenocortical insufficiency in our cases, despite the normal response to exogenous ACTH, raised the possibility that the secretion of endogenous adrenocorticotropic hormone had been suppressed by the prolonged administration of prednisone and/or ACTH. There is evidence that adrenal steroids suppress both the release (16) and biosynthesis of endogenous ACTH (17) while they are being administered. In addition, Kyle and his co-workers have suggested that in certain patients with a preceding history of spontaneous or iatrogenic Cushing's syndrome, functional impairment of endogenous ACTH secretion may be demonstrated and may persist for long periods of time (18, 19). Forsham *et al.* reported that if ACTH was abruptly discontinued in patients who had been treated with it for "prolonged periods of time" adrenocortical activity returned to, but did not fall below, the pre-ACTH baseline (20).

In the rat, administration of exogenous ACTH has been reported both to decrease (21) and increase (22) the content of ACTH in the pituitary and to inhibit the release of ACTH in adrenalectomized rats subjected to stress (23). Only limited data are available on the effects of simultaneous administration of steroids and ACTH in the rat. These data indicate that

both the pituitary content and release of ACTH are diminished during therapy but rapidly return toward normal after discontinuation of the hormones (24). In our own patients, the results obtained by the insulin tolerance test indicate that the hypothalamic-hypophyseal-adrenal system was intact after steroid-ACTH withdrawal, even though some of the patients were experiencing withdrawal symptoms. Therefore, we probably can assume that the synthesis and release of endogenous ACTH in response to stress was not impaired. That conclusion is supported by the results of measurement of 3 established parameters of hypophyseal-adrenal cortical function, *viz*, insulin sensitivity, presence of hypoglycemia responsiveness, and a rise in the level of plasma cortisol.

The finding that the withdrawal patients were significantly less sensitive to insulin than were the controls is of interest and merits speculation. Since some of the withdrawal patients had anorexia at the time of the insulin tolerance test, it seems possible that they may have been in a fasting state longer than the controls. If true, the secretion of insulin antagonists, such as growth hormone, in response to fasting may have altered the sensitivity to insulin and may also explain the somewhat abnormal tolerance to intravenous carbohydrate in some of the patients. However, even the withdrawal patients who were not anorectic tended to be less sensitive to insulin than were the controls. In none of the patients studied had diabetes mellitus developed during steroid-ACTH therapy. Other studies are necessary to clarify the situation.

The use of an acute brief stress such as insulin hypoglycemia and dependence upon a rise in plasma cortisol concentration as a measure of adequate hypothalamic-pituitary-adrenal cortical function might be criticized for a number of reasons. If the tissue requirement for cortisol were increased as an adaptation to a prolonged period of hyperadrenocorticism, a given "normal" rise in plasma cortisol concentration in response to stress might actually be "subnormal" for that particular patient. That theoretical possibility seems unlikely, since infused cortisol disappears from the plasma of patients with spontaneous (14) or induced hyperadrenocorticism (13) at a normal rate.

A more difficult criticism to counter is the possibility that, if only partial adrenocorticotropin insufficiency exists, the response to the brief acute stress of insulin hypoglycemia might be adequate even through the response to a sustained stress is inadequate. A surprising degree of pituitary reserve is normally present, as has been domonstrated in experiments employing the technique of graded hypophysectomy in the dog, since in that species 95 per cent of the adenohypophysis must be destroyed before adrenal atrophy occurs (25). This possibility is currently under investigation using other means of evaluating the hypothalamic-hypophyseal-adrenal

system. However, 2 of our patients did tolerate other major stress uneventfully. The patient who had a normal rise in plasma cortisol concentration during pulmonary surgery later tolerated surgical plication of a perforated peptic ulcer uneventfully in another hospital without steroid therapy. Another patient successfully tolerated an alcoholic debauch outside the hospital, accompanied by severe alcoholic gastritis, nausea, vomiting and virtual starvation for more than twenty-four hours, without steroid coverage.

The problem is also under investigation elsewhere. Holub *et al.* employing the adrenal 11-β-hydroxylase inhibitor, SU 4885, recently presented evidence that the suppressed hypothalamic-pituitary-adrenal system of patients receiving long-term steroid therapy almost invariably returns to normal after withdrawal of the steroid (26). Furthermore, they found a normal concentration of ACTH in the pituitaries of patients who died while receiving adrenocorticosteroid therapy. Since adrenocortical atrophy was present, exogenous steroid therapy in man appears to inhibit the release but not the biosynthesis of endogenous ACTH, in contrast to the rat in which both are impaired (24).

The symptoms that occurred in our patients after steroid-ACTH withdrawal clinically suggested the presence of adrenocortical insufficiency. They are difficult to explain in view of the laboratory evidence indicating an intact hypothalamic-pituitary-adrenocortical system. Since the symptoms were identical to those described after correction of spontaneous hyperadrenocorticism (1–3) it seems probable that some common mechanism is operative in both situations and that neither anterior pituitary nor adrenocortical insufficiency accounts for the syndrome.

Slocumb (4) described a "panmesenchymal reaction" in patients with rheumatic diseases during gradual withdrawal of steroid therapy, characterized by increased fatigability, myalgia, arthralgia and emotional instability. In some instances acute vasculitis (27), panniculitis (28) and peripheral neuritis (28) have developed during or after withdrawal of steroid therapy. Anorexia, nausea, lethargy and desquamation, which were so prominent in our steroid-ACTH withdrawal patients, have not been mentioned.

In view of the differing symptomatology, it is not clear whether the syndrome we observed is related to that described by Slocumb, Rotstein and Good, and others. However, it is possible that the differences may have been conditioned by the different types of patients studied, particularly if rheumatoid arthritis and other collagen diseases predispose to the development of vasculitis. Histologic evidence of vasculitis and panniculitis should be sought in patients without collagen disease who are experiencing symp-

toms after steroid withdrawal or after bilateral adrenalectomy for Cushing's syndrome. At the present time the etiology and pathogenesis of the hyperadrenocorticism-withdrawal syndrome remain to be elucidated.

It should be re-emphasized that the results obtained in this study, which employed both prednisone and ACTH, may not necessarily apply to patients treated with steroids alone in whom the sudden withdrawal of steroid therapy may be followed by true adrenal insufficiency. However, evidence is beginning to accumulate that even patients treated with steroids alone eventually attain a normal hypothalamic-pituitary-adrenal system after withdrawal of steroid therapy in the great majority of cases (26).

Acknowledgment

We are grateful to Dr. R. C. Merrill, Medical Director of Organon, Inc., for generous supplies of Cortrophin-Zinc.

REFERENCES

1. Sprague, R. G.; Kvale, W. F., and Priestley, J. T.: Management of certain hyperfunctioning lesions of the adrenal cortex and medulla, *J.A.M.A.* **151:** 629–639 (Feb. 21) 1953.
2. Cope, O., and Raker, J. W.: Cushing's disease; the surgical experience in the care of 46 cases, *New England J. Med.* **253:** 119–127 (July 28) 1955.
3. Skanse, B.; Gydell, K.; Wulff, H. B., and Koch, F.: Cushing's syndrome and its response to adrenalectomy, *Acta med. scandinav.* **154:** 119–134, 1956.
4. Slocumb, C. H.: Rheumatic complaints during chronic hypercortisonism and syndromes during withdrawal of cortisone in rheumatic patients, *Proc. Staff Meet., Mayo Clin.* **28:** 655–657 (Nov. 18) 1953.
5. Ingle, D. J., and Kendall, E. C.: Atrophy of the adrenal cortex of the rat produced by the administration of large amounts of cortin, *Science* **86:** 245 (Sept. 10) 1937.
6. Salassa, R. M.; Bennett, W. A.; Keating, F. R., Jr., and Sprague, R. G.: Postoperative adrenal cortical insufficiency; occurrence in patients previously treated with cortisone, *J.A.M.A.* **152:** 1509–1515 (Aug. 15) 1953.
7. Bondy, P. K.; Abelson, D.; Scheuer, J.; Tsen, T. K., and Upton, V.: Determination of cortisol in human plasma by quantitative paper chromatography, *J. Biol. Chem.* **224:** 47–55 (Jan.) 1957.
8. Fraser, R.; Albright, F., and Smith, P. H.: The value of the glucose tolerance test, the insulin tolerance test, and the glucose-insulin tolerance test in the diagnosis of endocrinologic disorders of glucose metabolism, *J. Clin. Endocrinol.* **1:** 297–306 (Apr.) 1941.
9. Somogyi, M.: Determination of blood sugar, *J. Biol. Chem.* **160:** 69–73 (Sept.) 1945.
10. Peterson, R. E.; Wyngaarden, J. B.; Guerra, S. L.; Brodie, B. B., and Bunim, J. J.: The physiological disposition and metabolic fate of hydrocortisone in man, *J. Clin. Invest.* **34:** 1779–1794 (Dec.) 1955.
11. Scheuer, J., and Bondy, P. K.: The effect of intravenous cortisol injections on the plasma cortisol concentration in man, *J. Clin. Invest.* **36:** 67–73 (Jan.) 1957.

12. ENGLEMAN, E. P.; KRUPP, M. A.; JOHNSON, H. P., JR.; WELSH, J. E.; WRENN, H. T., and KING W. R.: Adrenocortical function during continuous long-term therapy with cortisone, *A.M.A. Arch. Int. Med.* **91:** 1–7 (Jan.) 1953.
13. SANDBERG, A. A.; EIK-NES, K.; MIGEON, C. J., and KOEPF, G. F.: Plasma 17-hydroxycorticosteroids in hyperfunction, suppression, and deficiency of adrenal cortical function, *J. Lab. & Clin. Med.* **50:** 286–296 (Aug.) 1957.
14. CHRISTY, N. P.; LONGSON, D., and JAILER, J. W.: Studies in Cushing's syndrome. I. Observations on the response of plasma 17-hydroxycorticosteroid levels to corticotropin, *Am. J. Med.* **23:** 910–916 (Dec.) 1957.
15. YOUNG, I. I.; DEFILIPPIS, V.; MEYER, F. L., and WOLFSON, W. Q.: Maintenance of adrenal cortical responsiveness during prolonged corticoid therapy, *A.M.A. Arch. Int. Med.* **100:** 1–10 (July) 1957.
16. SAYERS, G., and SAYERS, M. A.: Regulation of pituitary adrenocorticotrophic activity during the response of the rat to acute stress, *Endocrinology* **40:** 265–273 (Apr.) 1947.
17. FARRELL, G. L., and LAQUEUR, G.: Reduction of pituitary content of ACTH by cortisone, *Endocrinology* **56:** 471–473 (Apr.) 1955.
18. KYLE, L. H.; MEYER, R. J., and CANARY, J. J.: Mechanism of adrenal atrophy in Cushing's syndrome due to adrenal tumor, *New England J. Med.* **257:** 57–61 (July) 1957.
19. CARREON, G. G.; CANARY, J. J., and KYLE, L. H.: Adrenocortical function after long-term corticoid therapy, (abstract) *Clin. Research* **7:** 146–147 (Jan.) 1959.
20. FORSHAM, P. H.; THORN, G. W.; FRAWLEY, T. F., and WILSON, L. W.: Studies on the functional state of the adrenal cortex during and following ACTH and cortisone therapy, (abstract) *J. Clin. Invest.* **29:** 812–813 (May) 1950.
21. GEMZELL, C. A., and HEIJKENSKJOLD, F.: Effect of corticotropin on the content of corticotrophin in the pituitary glands of adrenalectomized rats, *Acta endocrinol.* **24:** 249–254 (Mar.) 1957.
22. KITAY, J. I.; HOLUB, D. A., and JAILER, J. W.: Hormonal regulation of pituitary adrenocorticotrophin, *Proc. Soc. Exper. Biol. & Med.* **97:** 165–169 (Jan.) 1958.
23. KITAY, J. I.; HOLUB, D. A., and JAILER, J. W.: Inhibition of pituitary ACTH release: an extra-adrenal action of exogenous ACTH, *Endocrinology* **64:** 475–482 (Apr.) 1959.
24. HOLUB, D. A.; KITAY, J. I., and JAILER, J. W.: Effects of exogenous adrenocorticotrophic hormone (ACTH) upon pituitary ACTH concentration after prolonged cortisone treatment and stress, *J. Clin. Invest.* **38:** 291–298 (Feb.) 1959.
25. GANONG, W. F., and HUME, D. M.: The effect of graded hypophysectomy on thyroid, gonadal, and adrenocortical function in the dog, *Endocrinology* **59:** 293–301 (Sept.) 1956.
26. HOLUB, D. A.; JAILER, J. W.; KITAY, J. I., and FRANTZ, A. G.: Direct and indirect estimation of pituitary adrenocorticotropin reserves in man following steroid therapy, *J. Clin. Endocrinol. & Metab.* **19:** 1540–1552 (Dec.) 1959.
29. KEMPER, J. W.; BAGGENSTOSS, A. H., and SLOCUMB, C. H.: The relationship of therapy with cortisone to the incidence of vascular lesions in rheumatoid arthritis, *Ann. Int. Med.* **46:** 831–851 (May) 1957.
28. ROTSTEIN, J., and GOOD, R. A.: Steroid pseudorheumatism, *A.M.A.Arch. Int. Med.* **99:** 545–555 (Apr.) 1957.

STUDIES IN STEROID METABOLISM. IX. THE EXCRETION OF PREGNANE-3α,17α,20α-TRIOL DURING PREGNANCY*

FRANK F. RONAN, LANGDON PARSONS, M.D., ROBERT NAMIOT, A.B. AND HERBERT H. WOTIZ, PH.D.

The Department of Gynecology, Massachusetts Memorial Hospitals, and the Department of Biochemistry, Boston University, School of Medicine, Boston, Massachusetts

ABSTRACT

Evidence is presented that certain enzyme preparations are unable to hydrolyze pregnane-3α,17α,20α-triol, although they readily cleave pregnane-3α,20α,diol. Comparison of enzymatic hydrolysis with mineral acid hydrolysis showed that the latter caused conversion of pregnanetriol to a substance with chromatographic properties similar to those of pregnanediol, resulting in interference with the assay of pregnanediol. The excretion of pregnanetriol during pregnancy (7 cases) showed two major peaks—in the first and third trimesters. Between these peaks there was complete disappearance of pregnanetriol from the urine at about the twelfth week. This suggests that the site of production of pregnanetriol precursors had shifted from the ovary and possibly the adrenal cortex to the placenta.

THE excretion of pregnane-3α,20α-diol during pregnancy has been investigated many times since this steroid was first shown to be the primary metabolite of the placental hormone, progesterone. Although it was early recognized that pregnanediol occurred in urine largely as the glucuronate, it was not until the utilization of the enzyme β-glucuronidase for the cleavage of the conjugate that a relatively rapid type of assay, unhindered by the formation of acid-formed artifacts, could be devised for its quantitative analysis. Even then, one of the major problems was the adequate purification of pregnanediol prior to assay, which was usually accomplished by the formation of a sulfuric acid chromogen of low specificity. The excellent method described by Bongiovanni and Clayton (1) has put a tool into the hands of investigators which allows not only a fairly rapid assay of progesterone metabolites, but also a chemically specific one.

That the chromatographed hydrolysates obtained from the crude fraction of the "pregnanediol complex" were not homogeneous was already known (2). The work of Appleby and Norymberski (3) particularly indi-

Received July 24, 1959.

* These studies were supported by research grants from the National Institutes of Health, United States Public Health Service, Bethesda, Md.

cated that during pregnancy there appeared an increase in a fraction of urinary extracts which had the structure of a 17,20,21-triol or a 17,20-diol (as is found in pregnanetriol). Shortly afterwards, Hermann and Silverman (4) confirmed the observation that during the last trimester of pregnancy there is an increase in urinary pregnane-3α,17α,20α-triol.

In this communication it will be shown that pregnanetriol-glucuronate is significantly increased in the urine of pregnant females. This elevation begins about the fifth week of pregnancy; excretion then decreases to a low value about the twelfth week and again rises to a new maximum in the last trimester. It will also be shown that certain preparations of β-glucuronidase preferentially cleave the conjugate of pregnanediol without hydrolyzing the pregnanetriol, and that acid hydrolysis gives rise to falsely elevated pregnanediol values concomitant with the disappearance of the pregnanetriol fraction.

METHODOLOGY

For the routine determination of progesterone metabolites in urine the method of Bongiovanni and Clayton (1) was used with the following modifications:

1. Instead of 15,000 units of β-glucuronidase[1] per 50 ml. of urine, only 1500 units were added for each determination (Fig. 1).
2. Incubation was carried out for approximately seventeen hours (overnight).
3. The chromatographic column (11 mm. diameter) was packed with 9 Gm. of alumina.[2]
4. The concentration of ethanol in benzene for the second eluate was increased to 3 per cent.
5. The color produced by the reaction of the steroid with sulfuric acid was measured after standing overnight rather than after twenty minutes (5).
6. The correction factor for the determination of pregnanetriol was

$$D_{corr.} = \text{Absorbance at } 440\ m\mu - \frac{410\ m\mu + 470\ m\mu}{2},$$

as described by Bongiovanni and Eberlein (6).

RESULTS AND DISCUSSION

The work of Wotiz *et al.* (7) indicated that maximum recovery of 17-ketosteroids from 50 ml. of urine required about 2500 units of Ketodase. In contrast Bongiovanni and Clayton (1), using apparently the same commercial preparation, employed six times as much enzyme for the liberation of pregnanetriol from its conjugate. In Figure 1 are shown the results of three experiments using 9.5 mg. of crystalline sodium pregnanediol glucuronate following incubation with increasing concentrations of Ketodase

[1] Warner-Chilcott Ketodase.

[2] Harshaw Scientific Co.

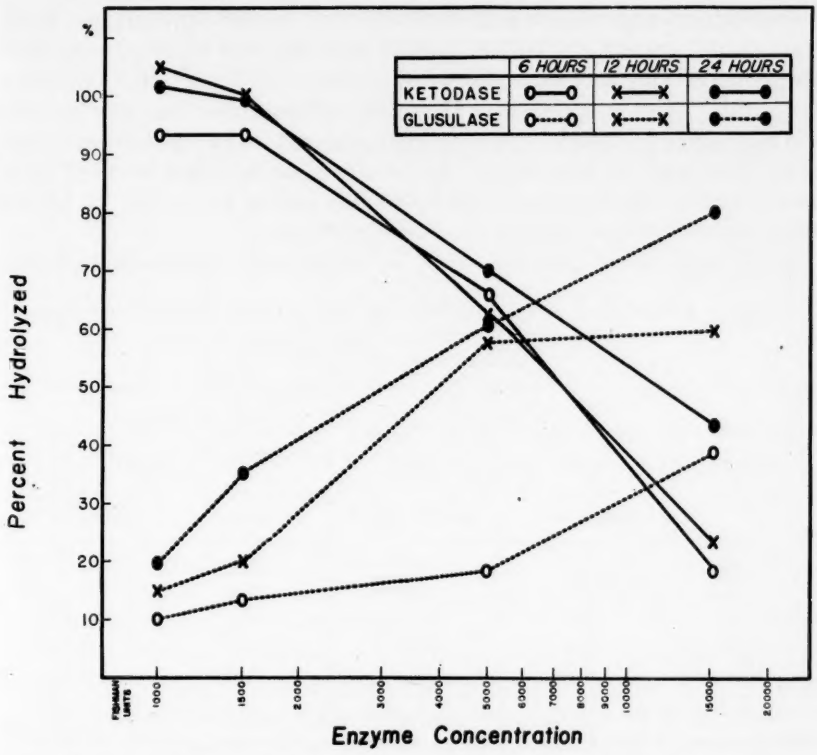


FIG. 1. Results of the hydrolysis of pregnanediol glucuronate by increasing amounts of Ketodase and Glusulase. Each curve represents the average recovery of pregnanediol in 3 individual experiments.

and another β-glucuronidase.[3] The experiments were carried out in an aqueous medium at 37°C. for six, twelve and twenty-four hours.

Maximum hydrolysis was obtained with 1500 units of Ketodase after even six hours. This compares favorably with the work on urinary 17-ketosteroids. Interestingly, there consistently occurred a marked decrease in the recovery of free steroid with the use of larger quantities of the enzyme Ketodase. In contrast, the lyophilized enzyme Glusulase never yielded maximum recoveries at six hours, but did show the expected increase in free steroid with increasing enzyme concentration and more prolonged incubation time.

[3] The authors are indebted to Dr. S. Gordon of Endo Products for contributing this enzyme. It is a lyophilized extract from *Helix pomatia* and is designated "Glusulase." This preparation contained 140,000 units of β-glucuronidase activity and 230,000 units of sulfatase activity per milliliter.

Preliminary experiments indicated that the two different enzyme preparations did not yield identical results, and that the levels of combined pregnanediol and pregnanetriol were consistently lower after Glusulase hydrolysis. Table 1 shows that Glusulase differentiated the two steroids and selectively hydrolyzed pregnanediol glucuronate to the total exclusion of the pregnanetriol derivative. The usually observed lower levels of pregnanediol after Glusulase treatment probably can be accounted for by the lower activity of this enzyme, as shown in Figure 1.

Since most of the previous work on pregnanediol determinations described in the literature utilized mineral acid hydrolysis (8–10), a series of pregnancy urines were analyzed for both metabolites following acid and enzymatic hydrolysis in serial aliquots. Table 2 shows that after acid treatment all the chromogenic material appeared in the 3 per cent alcohol fraction but none was found in the 10 per cent alcohol eluate.[4] From these data it appears that not only is there a complete disappearance of the pregnanetriol fraction with a concomitant increase in the pregnanediol portion, but that acid hydrolysis actually increases the "total preganediol" concentration to a value in excess of the sum of the pregnanediol and pregnanetriol values obtained by enzymatic cleavage. This may be accounted

TABLE 1. COMPARISON OF HYDROLYSIS WITH TWO DIFFERENT β-GLUCURONIDASE, PREPARATIONS USING URINE SPECIMENS FROM NORMALLY MENSTRUATING FEMALES

Subject	Glusulase*		Ketodase†	
	24-hr. excretion (mg.)		24-hr. excretion (mg.)	
	pregnanediol	pregnanetriol	pregnanediol	pregnanetriol
J.M.	1.0	0.0	1.2	0.5
N.B.	1.7	0.0	1.8	3.0
D.R.	0.4	0.0	0.7	1.2
J.M.	1.0	0.0	1.2	0.7
L.S.	3.6	0.0	3.0	0.3

* Glusulase is a lyophylized preparation of *Helix pomatia* extract obtained from Endo Products. A 50-ml. sample of urine was incubated for 24 hours with 15,000 units of glucuronidase activity at pH 5.2.

† Ketodase is a commercially available preparation (Warner-Chilcott Laboratories). A 50-ml. sample of urine was incubated with 1500 units for 17 hours at pH 5.2.

[4] The nature of the 10 per cent ethanol fraction from a urine not treated with acid was investigated following further paper and alumina column chromatography by means of infrared spectroscopy. The spectrum of the primary substance thus isolated —two minor fractions were seen but not identified—was identical with that of an authentic specimen of pregnanetriol.

TABLE 2. COMPARISON OF HYDROLYSIS WITH MINERAL ACID AND WITH KETODASE, USING URINE SPECIMENS FROM PREGNANT WOMEN

Patient	Week of pregnancy	HCl*		Ketodase		
		24-hr. excretion (mg.)		24-hr. excretion (mg.)		
		3% EtOH fraction†	10% EtOH fraction‡	3% EtOH fraction	10% EtOH fraction	Sub-total
B.L.	30	28.0	0.0	16.3	6.1	22.4
A.K.	29	45.9	0.0	29.4	5.1	34.5
J.J.	33	61.4	0.0	32.4	4.8	37.2
J.H.	32	62.1	0.0	34.2	7.2	41.4
M.B.	24	26.1	0.0	18.0	3.9	21.9

* Hydrochloric acid hydrolysis was carried out by boiling the urine specimen with 15 vol. % of conc. HCl for thirty minutes.

† Pregnanediol.

‡ Pregnanetriol.

for either by the release of nonspecific sulfuric acid reactive chromogens, or by a markedly higher absorption coefficient of the acid-altered material.

The conversion of pregnanetriol by hot mineral acid to a substance which was eluted with 3 per cent ethanol in benzene was further demonstrated by the addition of synthetic pregnanetriol to urine, acid hydrolysis of the mixture, and by routine assay of the proper fractions. In Table 3 are the data showing that 65 per cent of the pregnanetriol added to a specimen of urine from a patient with panhypopituitarism was recovered following enzymatic treatment; following acid hydrolysis, on the other hand, all of the pregnanetriol appeared in the 3 per cent ethanol (pregnanediol) fraction and none was eluted with 10 per cent ethanol in benzene.

TABLE 3. RECOVERY OF PREGNANETRIOL FROM URINE AFTER TREATMENT WITH KETODASE AND AFTER TREATMENT WITH HCl

Sample	Recovery of steroid* after treatment of urine with			
	HCl		Ketodase	
	3% EtOH fraction	10% EtOH fraction	3% EtOH fraction	10% EtOH fraction
Urine†			0.2 μg.	0.0 μg.
Urine plus steroid	113%	0.0%	0.0%	65%

* 80 μg. of spectroscopically pure pregnanetriol was added to the urine.

† The urine specimen was obtained from a patient with panhypopituitarism.

It has been previously shown by Fukushima and Gallagher (11) that pregnanetriol is degraded by mineral acid, resulting in dehydration at C-17 followed by rearrangement to pregnanolone in approximately 13 per cent yield. This latter substance, however, is eluted in the 0.5 per cent ethanol eluate and, therefore, can not account for the enhancement of the pregnanediol fraction. The behavior of this new substance on the chromatographic column and the absorption maxima suggest that this material is of greater polarity and differs in structure from pregnanolone. It may well be one of several unidentified side-products obtained by these authors.

Figure 2 shows the urinary excretion of pregnanediol and pregnanetriol in a normal female from whom urines had been fortuitously collected prior to knowledge of conception, allowing a study of steroid excretion over the course of a complete pregnancy. The average level of excretion of pregnanetriol was about 4 mg. per twenty-four hours with a peak of 7.4 mg. during the first trimester. There was an abrupt decrease in urinary pregnanetriol during the eleventh week which was not duplicated but was paralleled a week later by pregnanediol. Subsequently there was a second rise in the

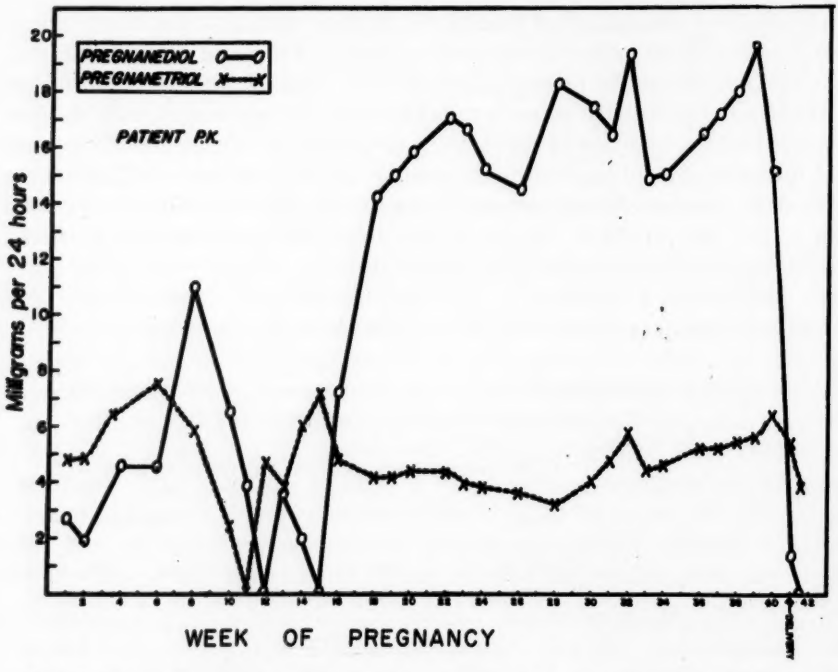


FIG. 2. Excretion of pregnanediol and pregnanetriol by a pregnant female. (The 24-hour specimens of urine were carefully collected once a week by a sympathetic former collaborator.)

excretion of both compounds. The peak height for pregnanediol was greater during the second rise, but that for pregnanetriol was about the same. At parturition the expected drop in progesterone metabolites was observed.

The most interesting feature was the sharp decrease in pregnanediol and pregnanetriol excretion near the end of the first trimester. In order to rule out the possibility of individual variation or experimental error, several other pregnancy urines were analyzed before, on or near, and after the eleventh week. In Table 4 are recorded the results obtained from 7 such

TABLE 4. EXCRETION OF PREGNANEDIOL AND PREGNANETRIOL DURING PREGNANCY

Patient	Age (yrs.)	Week of pregnancy	Urinary excretion (mg./24 hrs.)	
			Pregnanediol	Pregnanetriol
P.K.	27	6	4.6	7.5
		11	3.9	0.0
		12	0.0	4.8
		32	19.2	6.0
B.Z.$_1$	25	6	3.2	6.5
		10	1.2	0.2
		11	0.0	5.2
		32	17.2	7.3
L.L.	27	7	7.3	7.9
		13	0.0	1.4
		29	18.6	6.3
L.S.	31	6	9.8	8.1
		12	1.0	3.1
		32	22.2	9.3
		36	27.0	7.1
A.B.	29	5	5.9	7.2
		14	0.0	2.1
		33	27.0	8.6
G.K.	28	7	3.2	6.4
		13	0.9	4.2
		31	18.5	3.6
B.Z.$_2$	26	5	10.8	5.1
		9	8.2	0.4
		10	0.8	3.3
		11	3.2	3.9
		12	6.5	5.8
		18	14.8	6.2

investigations. The great difficulty in getting a sufficient number of specimens from each patient prevented a complete study over all the weeks of pregnancy. Nevertheless, 24-hour urine collections were obtained close to what appears to be the critical time for the transitory decrease in the excretion of both steroids. Six out of 7 patients showed either a complete lack of, or a markedly decreased excretion of, pregnanetriol between the tenth and the fourteenth week. A similar decrease was also found for the excretion of pregnanediol.

From the data on the first, second and seventh patients of Table 4, as well as from the data of Figure 2, it appears that the drop in pregnanetriol excretion precedes the temporary disappearance of pregnanediol. This suggests that the decrease in pregnanetriol occurs earlier than the twelfth week rather than later, and might account for the nonconformity of Patient G.K. whose urinary pregnanediol was very low in the thirteenth week. It is probable then that the lowest excretion of pregnanetriol by this patient occurred in week 11 or 12. Although Patients L.L., L.S. and A.B. showed marked decreases in pregnanetriol excretion during weeks 13, 12 and 14 respectively, the fact that their pregnanediol excretion values at these times were at or near zero again suggests that the critical collection time was missed by approximately a week.

It is thought that this biphasic curve for the excretion of progesterone metabolites may reflect a change in the site of formation of this compound and of 17α-hydroxyprogesterone. The latter substance is on the biosynthetic pathway to the corticosteroids and is formed from progesterone. Hydroxylations at positions 11 and 21 are all that is necessary to produce the most active naturally occurring adrenocorticosteroid. It is therefore suggested that shortly after conception there is an increase in production of both these hormones by the ovary and possibly by the adrenal cortex, which abates toward the end of the first trimester; at this time the placenta, having reached sufficient size, takes over the function of producing a corticosteroid precursor.

REFERENCES

1. Bongiovanni, A. M., and Clayton, G. W., Jr.: A simplified method for the routine determination of pregnanediol and pregnanetriol in urine, *Bull. Johns Hopkins Hosp.* **94:** 180, 1954.
2. Smith, O. W., and Smith, G. V.: Endocrinology and related phenomena of the human menstrual cycle, *Recent Progr. Hormone Res.* **7:** 209, 1952.
3. Appleby, J. I., and Norymberski, J. K.: The urinary excretion of 17-hydroxycorticosteroids in human pregnancy, *J. Endocrinol.* (London) **15:** 310, 1957.
4. Herrmann, W., and Silverman, L.: Excretion of pregnane-3α,17α,20α-triol in pregnancy, *J. Clin. Endocrinol. & Metab.* **17:** 1482, 1957.
5. Verboom, E.: Quantitative determination of pregnane-3α,20α-diol in the urine, *Acta endocrinol.* **24:** 1, 1955.

6. Bongiovanni, A. M., and Eberlein, W. R.: Critical analysis of methods for measurement of pregnane-3α,17α,20α-triol in human urine, *Analyt. Chem.* **30**: 388, 1958.
7. Wotiz, H. H.; Lemon, H. M.; Marcus, P., and Savard, K.: The conjugated 17-ketosteroids of human urine, *J. Clin. Endocrinol. & Metab.* **17**: 534, 1957.
8. Huber, D.: Determination of pregnanediol in urine for diagnostic purposes, *Biochem. J.* **41**: 609, 1947.
9. de Watteville, H.: Pregnanediol determinations in the clinic and in research, *J. Clin. Endocrinol.* **11**: 251, 1951.
10. Rogers, J., and Sturgis, S. H.: Pregnanediol excretion in normal women, *J. Clin. Endocrinol.* **10**: 89, 1950.
11. Fukushima, D. K., and Gallagher, T. F.: Metabolism of 21-deoxyhydrocortisone-4-C^{14} in man, *J. Biol. Chem.* **266**: 725, 1957.

THE NORMAL RANGE OF SERUM INORGANIC PHOSPHORUS AND ITS UTILITY AS A DISCRIMINANT IN THE DIAGNOSIS OF CONGENITAL HYPOPHOSPHATEMIA*

BERNARD G. GREENBERG, PH.D.,† ROBERT W. WINTERS, M.D.‡ AND JOHN B. GRAHAM, M.D.§

The Department of Biostatistics, School of Public Health, and the Departments of Medicine, Pediatrics, and Pathology, School of Medicine, University of North Carolina, Chapel Hill, N. C.

ABSTRACT

The range of serum inorganic phosphorus was determined (Fiske and SubbaRow method) on a normal population of 908 persons. This was accomplished by fitting complex curvilinear functions of phosphorus on age separately for each sex. These functions were deemed necessary, because it became apparent that serum phosphorus concentration varied with both age and sex. Ninety-nine per cent confidence limits were placed on the normal functions, and the lower limit of this confidence belt was used as a discriminant for the diagnosis of familial hypophosphatemia. Similar functions were fitted separately by sex to a population of 70 affected persons from five families segregating for familial hypophosphatemia. It was found by studying the overlap between the confidence limits of the normal and abnormal populations that the lower 99 per cent confidence limit of the normal function was a superior diagnostic tool. The true operating characteristics of this discriminant were then determined. This required a general mathematical solution of the problem of evaluating a discriminant when the populations used for the comparison contain persons with incorrectly diagnosed disease, and there is also an error associated with the chemical determination.

A SEARCH of the available literature reveals that the "generally accepted" normal values for serum inorganic phosphorus concentration have never been rigorously established. Furthermore, the literature does not contain sufficient published data for calculating norms with suitable confidence limits. It became necessary for us to establish the "normal

Received August 10, 1959.

* Supported (in part) by grants from the United Medical Fund of North Carolina and the U. S. Public Health Service (H-1333 and H-3140).

† Professor of Biostatistics, School of Public Health, University of North Carolina.

‡ Formerly Post-doctoral Research Fellow of the National Foundation for Infantile Paralysis. Presently Assistant Professor of Physiology, University of Pennsylvania, Philadelphia, Pa.

§ Professor of Pathology, School of Medicine, University of North Carolina.

range" in order to evaluate serum phosphorus values in kindreds segregating for "uncomplicated" hypophosphatemia and vitamin D resistant rickets (1–3).

It has been generally recognized that infants and children have higher serum inorganic phosphorus levels than adults (4), but the relationship between serum phosphorus and age has been studied by only a few workers (5–8). Possible differences between sexes with respect to serum phosphorus concentration have also escaped general recognition, although some investigations have suggested such a difference (8, 9).

In the present study regression curves of serum phosphorus on age were fitted to a large number of normal observations. Functions were fitted separately by sex when inspection suggested that there was an effect of sex as well as of age. The reliability of the suggested normal limits is described, particularly as these limits apply to the diagnosis of hypophosphatemia in kindreds segregating for "uncomplicated" vitamin D resistant rickets. Finally, a general method is described in the Appendix for evaluating the operating characteristics of this type of discriminant despite errors in clinical diagnosis and in the laboratory method itself.

MATERIALS AND METHODS

Serum inorganic phosphorus values used in determining normal range

The phosphorus values used in fitting the regression curves were gathered from the literature (7, 8, 10) and supplemented by a large number of unpublished observations kindly supplied by several investigators (11–13). For inclusion in the normal population a subject could not be obviously abnormal, and his serum phosphorus level must have been determined by the method of Fiske and SubbaRow (14). Fortunately, most of the phosphorus determinations had been made on subjects in the basal state. No attempt was made to adjust for other possible influences such as season, menstruation, time of day, inter-laboratory variation, or vitamin D intake, because this information was usually not available.

Serum inorganic phosphorus values were obtained on 511 normal males and 397 normal females older than 1 year of age. Infants under this age were not included, because it is known that their serum phosphorus values are greatly affected by such additional variables as dietary phosphorus load (*e.g.*, breast milk versus cow's milk), and renal and parathyroid immaturity (15–20).

The precision or consistency of application of the phosphorus method was determined *within* each of the two laboratories furnishing the bulk of the normal data (11, 12). For females under 16 years of age, the residual variance about regression curves fitted separately by sex and laboratory was 0.002657 for one and 0.002367 for the other. These values are not significantly different and are, in fact, remarkably similar. This finding implies that the Fiske and SubbaRow method was managed systematically and comparably *within* each laboratory.

A difference was then sought between the phosphorus values for children under 16 studied in the two laboratories. Analysis showed that the average serum phosphorus level of a child in Iowa was slightly but significantly higher than that of a comparable

child in North Carolina. It was not possible to determine whether this difference resulted from a difference in nutritional status or a systematic difference in application of the phosphorus method. As pointed out subsequently, a systematic difference in a laboratory manipulation such as variation in the length of time filtrates are incubated after addition of the color reagent is sufficient to explain a difference of this magnitude.

Serum inorganic phosphorus determinations in our laboratory

Serum phosphorus levels were determined in our laboratory by our modification of the Fiske and SubbaRow method as described subsequently. The procedure was applied to 70 persons with vitamin D resistant rickets and familial hypophosphatemia who belonged to five kindreds segregating for this disorder (1–3). Multiple determinations were performed on most of the sera; all of the information on each subject was averaged, and the average was used as a single item in the statistical analysis.

Our modification of the Fiske and SubbaRow method was the following: Blood was carefully collected by venipuncture and allowed to clot in a conical centrifuge tube at room temperature. The clot was carefully freed, and the serum expressed by centrifugation. The serum was removed and *stored at 4° C. no longer than forty-eight hours; it was never frozen and thawed.* A 0.5-ml. sample of serum was pipetted into a 15-ml. conical centrifuge tube. Then 4 ml. of 20 per cent trichloracetic acid was added with gentle shaking. To this was added 8.0 ml. of distilled water. The solutions were mixed, allowed to stand for ten minutes, and centrifuged. Then 4.0 ml. of the filtrate (or standard) was pipetted into a suitable cuvette; into the cuvette were added, successively, 1.7 ml. of 0.5 *N* sulfuric acid, 0.2 ml. of aqueous ammonium molybdate and 0.1 ml. of aminonaphtholsulfonic acid reagent. The contents of the cuvette were mixed, and read exactly ten minutes later in the Coleman Junior spectrophotometer at 660 mμ.

Standard solutions of monobasic potassium phosphate (KH_2PO_4) were run with each set of determinations. These corresponded to serum samples containing 2, 3, 4, 5 and 10 mg. of phosphorus per 100 ml. The standard solutions contained the same final concentration of trichloracetic acid as the serum samples. In an occasional sample, cloudiness developed either in the filtrate or after adding the color reagents; such samples were always discarded.

(The aminonaphtholsulfonic acid reagent was prepared by mixing 5.8 Gm. of sodium bisulfite, 0.100 Gm. of sodium sulfite (anhydrous), and 0.100 Gm. of 1,2,4 aminonaphtholsulfonic acid (powder). These chemicals were dissolved in 40 ml. of distilled water by shaking, then filtered through phosphorus-free filter paper. Both the aminonaphtholsulfonic acid and the ammonium molybdate reagents were freshly prepared every two weeks.)

The error of our application of this phosphorus method was assessed by determining the levels in the sera of 10 presumably normal persons. The serum specimens were coded to assure anonymity and randomly assorted. Triplicate samples were withdrawn from each specimen. Two of the replicates were carried through the test simultaneously, and the third was allowed to stand as a protein-free filtrate for twenty additional minutes. The color intensity of the first two replicates was read twice, ten minutes and twenty-five minutes after addition of the color reagent. The color intensity of the third replicate was read after exactly ten minutes of color development.

Study of the results by analysis of variance showed that the first two readings were identical within the limits of experimental error of the test (see following section). The extra delay of fifteen minutes in reading the color intensity resulted in a significant decrease in the presumed value of phosphorus. The additional twenty-minute delay be-

tween centrifugation of the protein-free filtrate and addition of the color reagent made no difference.

The experimental error of the test itself was such that the coefficient of variation (standard deviation/mean) was only 1.2 per cent. The average standard deviation for our 10 test samples was equal to 0.044, which meant that a single determination had a 95 per cent probability of being within ±0.1 mg. per 100 ml. of the observed value, over the range of phosphorus values under study.

RESULTS

Determination of zones of normalcy for serum phosphorus concentration

Serum inorganic phosphorus values of 511 normal males and 397 normal females were plotted against age, separately by sexes. The patterns for each sex clearly indicated an effect of age on the serum phosphorus level. A variety of types of curves were fitted to the data by the method of least squares. The best fitting curve among those tested was of the general type:

$$\log_{10} y = a + bx + cx^2 + d \log_{10} x$$

where y = serum inorganic phosphorus in mg. per 100 ml., and x = age in years.

The equations fitted to each sex were:

Males: $\log_{10} y = 0.70824 - 0.007974x + 0.000061x^2 + 0.033718 \log_{10} x$

Females: $\log_{10} y = 0.73072 - 0.009610x - 0.000102x^2 + 0.016700 \log_{10} x.$

The phosphorus values and the regression curves are shown in Figure 1 for males and in Figure 2 for females. In each figure, the solid curves marked AVE. represent the average normal values at any age. Both curves at age 1 year indicate a phosphorus level in the neighborhood of 5 mg. per 100 ml., the curve for females being somewhat higher than that for males. The curve for males falls steadily and progressively for the first three decades after which it gradually flattens out, with only a slight up-turn after the age of 70 years. The curve for females, on the other hand, shows a steeper fall than the male curve during the earlier decades, reaching a definite minimum at about the age of 45 years. Thereafter, there is a slight, progressive up-turn.

Zones of normalcy representing 99 per cent confidence limits were calculated on the basis of the normal regression curves for each sex, using the variance expression for this type of function:

$$\sqrt{V(\log_{10} \hat{y})} = 2.576(s_{\log y \cdot x, x^2, \log_{10} x}) \times \sqrt{1 + \frac{1}{N} + 6 \text{ correction terms for imprecision in estimating } b, c \text{ and } d.}$$

The 99 per cent confidence limits are shown by the dashed lines marked

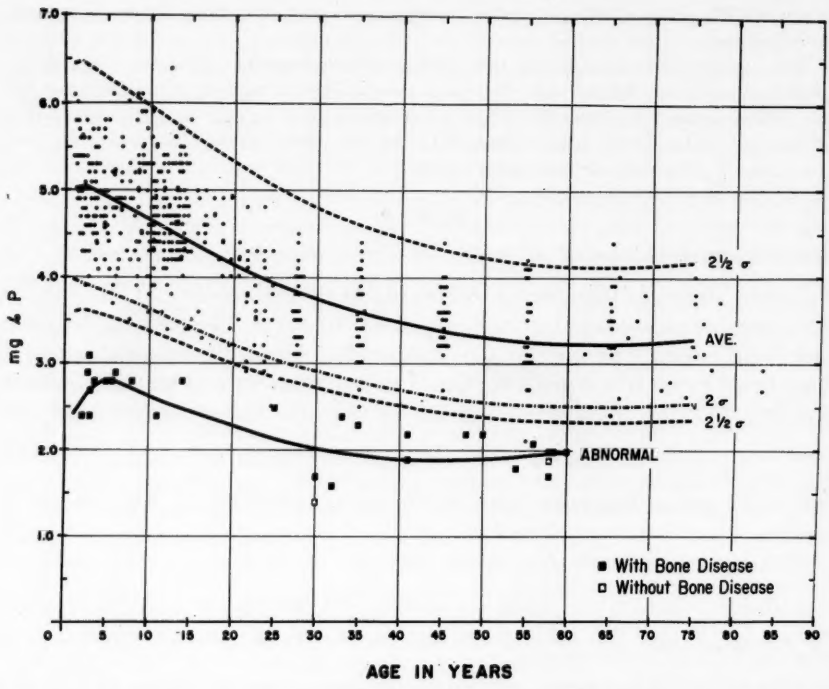


FIG. 1. Regression curves of serum inorganic phosphorus on age for *males*. The solid curve marked AVE. was fitted to the phosphorus values of 511 normal males, individually recorded as small black dots (•). The broken lines designated $2\frac{1}{2}\sigma$ are 99 per cent confidence limits for this normal control population. The broken line marked 2σ is the lower 95 per cent confidence limit. The solid black squares (■) are the serum phosphorus values of 27 males with clinical evidence of rickets belonging to 5 kindreds segregating for familial hypophosphatemia. The open squares (□) are hypophosphatemic males without clinical rickets from the same families. The solid curve marked ABNORMAL is of the same general type as the one marked AVE. and was fitted to the hypophosphatemic population. (See text for details.)

$2\frac{1}{2}\sigma$ in Figures 1 and 2. (The lower 95 per cent confidence limit is also shown. This is denoted as 2σ, and is added for the convenience of workers desiring a less stringent diagnostic standard.)

Determination of zones of abnormalcy

The serum phosphorus values of 70 abnormal subjects (29 males and 41 females) from five personally studied kindreds with vitamin D resistant rickets and familial hypophosphatemia were also plotted separately by sexes, as shown in Figures 1 and 2. A general relationship between age and

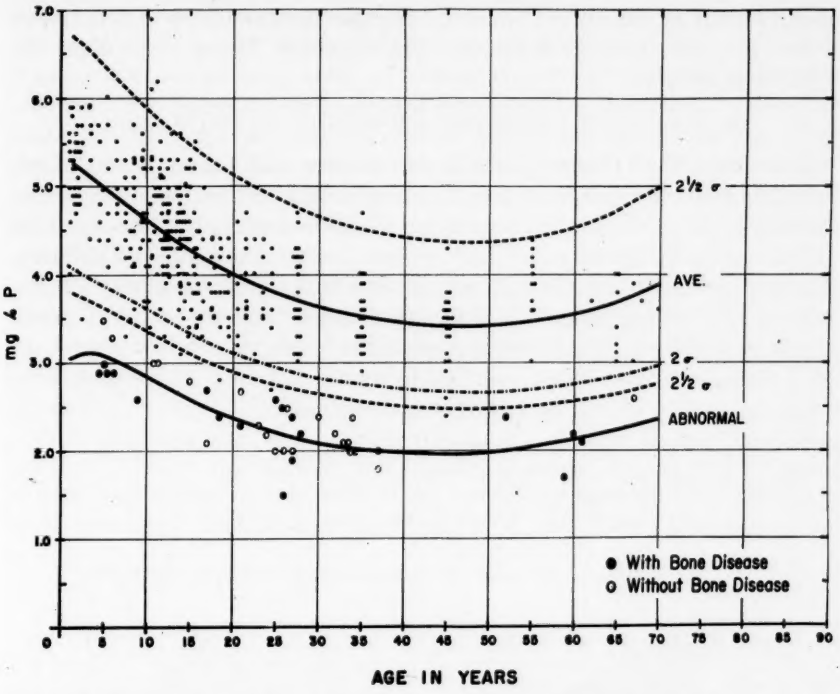


FIG. 2. Regression curves of serum inorganic phosphorus on age for *females*. The small black dots (•) represent the normal control population, and the larger symbols (●, ○) represent the hypophosphatemic women from the 5 affected families. The curves and symbols have the same meaning as in the legend for Figure 1.

serum phosphorus concentration, similar to that of the normal population, is apparent on inspection.

A regression curve of phosphorus on age of the general type previously described was fitted to the data from 29 males (Fig. 1). Of these 29 males, 27 could be considered clearly abnormal by independent clinical, chemical and radiologic criteria, as outlined elsewhere (2). The boys had either active bone disease or deformities typical of vitamin D resistant rickets, and the men had postrachitic deformities. The 2 males who were not clearly abnormal by other criteria were of short stature. One of these men had close relatives (mother, siblings, nieces and nephews) who were affected. The other man's position in the pedigree would have permitted him to be affected, but no other closely related person had rickets. Since the serum phosphorus level of these 2 men did not differ clearly from that of the larger group with bone disease or deformities, they were included in the abnormal

group for fitting the curve. These are the men assumed later (Table 1A) to represent false positive diagnoses. The equation fitting the values for *affected males* was:

$$\log_{10} y = 0.40327 - 0.015946x + 0.000157x^2 + 0.165764 \log_{10} x.$$

Only 19 of the 41 hypophosphatemic females had independent clinical, radiologic, or chemical evidence of active rickets, or had postrachitic deformities. An additional 12 could be considered probably abnormal on genetic grounds, because they had normal husbands but affected children. The remaining 10 had affected parents or siblings and hypophosphatemia *without* affected children. As in the case of males, the data on the clinically unaffected women whose serum phosphorus levels were below the 99 per cent confidence limit (later assumed to represent false positive diagnoses) were included in fitting the *curve for affected females:*

$$\log_{10} y = 0.49845 - 0.015067x + 0.000161x^2 + 0.090408 \log_{10} x.$$

Ninety-nine per cent confidence limits were also placed on the curve of each abnormal population. When these curves and their confidence limits

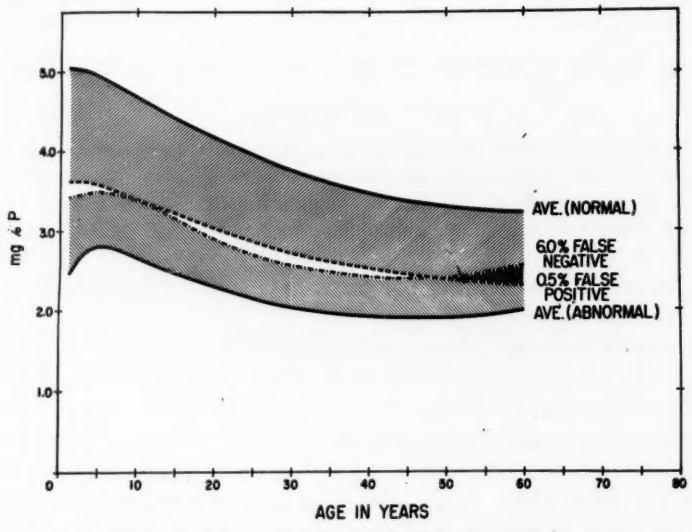


FIG. 3. Assumed operating characteristics of the lower 99 per cent confidence limit for the serum inorganic phosphorus concentration of normal men in the diagnosis of familial hypophosphatemia in *males*. The upper solid curve and the upper broken curve (– – –) are the normal curve for men with its lower 99 per cent confidence limit, taken from Figure 1. The lower solid curve is the ABNORMAL curve from Figure 1. The lower broken curve (–·–·) is the upper 94 per cent confidence limit for the abnormal population of males in the 5 families studied.

were plotted on the same graphs with the normal curves, it was seen that the zones of normalcy and abnormalcy overlapped considerably. An estimate of the false negative rate (*i.e.*, scored as normal but actually affected) was made by observing the degree of overlap of the normal and abnormal distributions when the lower limit for the normal population and the upper limit for the abnormal population were held constant at the 99 per cent point. It was found that the false negative rate varied somewhat with age, but averaged about 6 per cent for males and about 9 per cent for females over the entire age range.

Figures 3 and 4 are plotted to show the lower limits of the zones of normalcy (99 per cent confidence limits) and the upper limits of the zones of abnormalcy when the upper confidence limit for the abnormal males is held at 94 per cent (6 per cent false negative rate), and that for abnormal females at 91 per cent (9 per cent false negative rate).

It is apparent from these graphs that at certain ages there is still some overlap between the normal and abnormal zones. Among young males

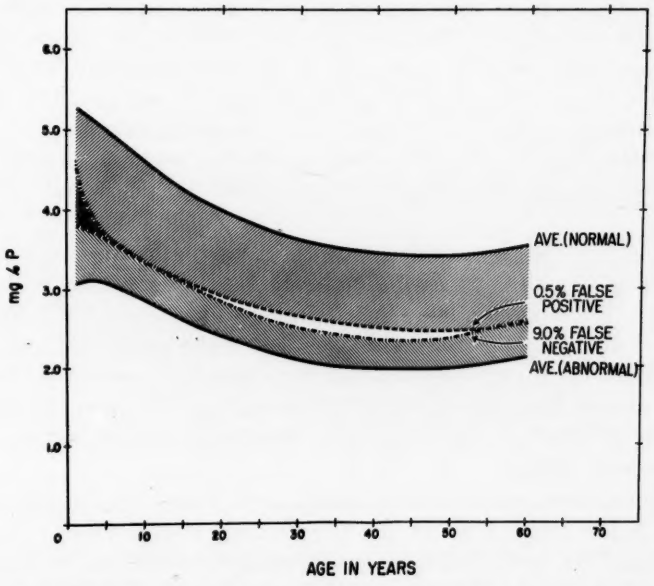


FIG. 4. Assumed operating characteristics of the lower 99 per cent confidence limit for the serum inorganic phosphorus concentration of normal women in the diagnosis of familial hypophosphatemia in *females*. The upper solid curve and the upper broken curve (– – –) are the normal curve for women with its lower 99 per cent confidence limit, taken from Figure 2. The lower solid curve is the ABNORMAL curve for females, also taken from Figure 2. The lower broken curve (–·–·) is the upper 91 per cent confidence limit for the abnormal population of females in the 5 families studied.

(Fig. 3) the chance of a false negative reading is less than 6 per cent, whereas in older men, when the two areas overlap (50–60 years), the false negative rate is greater than the average of 6 per cent. With the females, on the other hand (Fig. 4), the probability of an individual false negative reading at the younger ages may be as high as 15 per cent; thereafter, it is much less, averaging about 9 per cent.

Reliability of the lower 90 per cent confidence limit of the normal population as a discriminant for vitamin D resistant rickets

The determination of the foregoing zones of normalcy for the concentration of serum inorganic phosphorus were based on analyses of serum from groups of cases in which the diagnosis of vitamin D resistant rickets was not excluded. The data were collected by workers who rejected subjects as abnormal only when this was obvious from their appearance, and very few of these supposedly normal persons underwent intensive diagnostic study. Thus, the group of 908 presumably normal subjects may have contained a few abnormal persons who were improperly considered normal because of incomplete examination. It is our clinical impression that the general population contains fewer than 1 per cent undiscovered cases of familial hypophosphatemia and vitamin D resistant rickets. For our purposes, however, we assumed that exactly 1 per cent of the normal group used consisted of persons with this disorder, erroneously scored as normal. This is a conservative maximum value and amounts to 5 of the 511 males and 4 of the 397 females.

Similarly, it may be assumed that the abnormal groups contained normal persons erroneously scored as hypophosphatemic. Among certain members of the abnormal kindreds the clinical condition could not be diagnosed as clearly abnormal by criteria independent of the serum phosphorus level. We assumed for our calculations that these persons represent the false positive diagnoses. Hence, 2 of the 29 hypophosphatemic males and from 10 to 22 of the 41 females, depending upon diagnostic criteria, were considered to have been normal.

These assumptions have been summarized separately by sexes in Table 1. Here in the 2×2 tables, 1A and 1B, the truly normal subjects in the control population, assuming 1 per cent diagnostic error, are recorded in the upper left data cells. The upper right cells contain the abnormal persons erroneously included in the normal population on the same assumption of a 1 per cent diagnostic error. The lower right cells contain the members of the affected kindreds who were clearly abnormal on grounds independent of the serum phosphorus level.The lower left cells contain those members of the affected kindreds whose serum phosphorus levels were lower than the

TABLE 1. THE POSSIBLE FALSE POSITIVE AND FALSE NEGATIVE RATES IN THE CLINICAL DIAGNOSIS OF FAMILIAL HYPOPHOSPHATEMIA AND VITAMIN D RESISTANT RICKETS

A. *Males*

Presumed diagnosis	True diagnosis		
	Normal	Abnormal	Total
Normal	506	5	511
Abnormal	2	27	29
Total	508	32	540

B. *Females*

Presumed diagnosis	True diagnosis		
	Normal	Abnormal	Total
Normal	393	4	397
Abnormal	22 [10]*	19 [31]*	41
Total	415 [403]*	23 [35]*	438

C. *Rates—Both sexes*

Diagnosis	Males	Females
Possible false positive (α_0)	2/508 = 0.4%	22/415 = 5.3% (10/403 = 2.5%)*
Possible false negative (β_0)	5/32 = 15.6%	4/23 = 17.4% (4/35 = 11.4%)*

* Number if genetic criteria also considered.

lower 99 per cent confidence limit of the normal population, but who could not be considered affected on independent grounds.

The possible rates of false positive and false negative diagnosis, based on the data in the cells of sections 1A and 1B are shown in Table 1C.

Thus, the operating characteristics of the zones of normalcy and abnormalcy, assumed as shown in Figures 3 and 4, are 0.5 per cent false positive for both sexes, 6 per cent false negative for males, and 9 per cent false

negative for females. It becomes important to assess the impact upon these assumed operating characteristics of the possible false negative diagnostic rate (β_0) in classifying the normal group and the possible false positive diagnostic rate (α_0) in classifying the abnormal group.

The effects of the possible errors in diagnosis on these assumed operating characteristics were determined mathematically. The expressions derived for this purpose (see Appendix) were:

$$\text{True rate of false positive diagnosis,} \quad \alpha' = \alpha\left(\frac{p}{p - \alpha_0 p + \beta_0 r}\right)$$

and

$$\text{True rate of false negative diagnosis,} \quad \beta' = \beta\left(\frac{r}{r + \alpha_0 p - \beta_0 r}\right)$$

where α (false positive rate) and β (false negative rate) are the assumed operating characteristics of the diagnostic index; α_0 and β_0 are the possible false positive and false negative rates respectively in selecting the controls and patients originally; p is the proportion of persons in the general population not having the disease; r is the proportion having the disease; and α' and β' are the true operating characteristics of the diagnostic index.

The values of α' and β' calculated for several possible values of p and r for both sexes are shown in Table 2. In addition to the 3 possible values for r

TABLE 2. A TEST OF THE IMPACT OF THE POSSIBLE FALSE POSITIVE AND FALSE NEGATIVE DIAGNOSTIC RATES ON THE ASSUMED OPERATING CHARACTERISTICS OF SERUM PHOSPHORUS CONCENTRATION AS A DISCRIMINANT IN FAMILIAL HYPOPHOSPHATEMIA AND VITAMIN D RESISTANT RICKETS

A. *Males*

α_0	β_0	α	β	p	r	α'	β'
0.004	.156	0.005	.060	.999	.001	.005	.013
				.990	.010	.005	.048
				.950	.050	.005	.065

B. *Females*

α_0	β_0	α	β	p	r	α'	β'
0.053	.174	.005	.090	.999	.001	.005	.004
				.990	.010	.005	.014
				.950	.050	.005	.049
.025	.114	.005	.090	.999	.001	.005	.004
				.990	.010	.005	.028
				.950	.050	.005	.066

and p, two sets of values for α_0 and β_0 were tested for females, one set (.053 and .174) being the figures based upon completely independent clinical criteria, and the other (.025 and .114) those based on the combination of clinical and genetic criteria.

Under all assumptions for p, r, α_0 and β_0, the true rate of false positive diagnosis (α') remained in the neighborhood of 0.5 per cent. When the value for r is small, as it almost certainly is in the general population, the values for β' are also quite small. If the value for r were actually as large as 5 per cent of the population, the maximum rate of false negative diagnosis (β') would be 6.5 per cent for males and 6.6 per cent for females. Inasmuch as r is in reality probably much less than the maximum assumption of 5 per cent it is likely that β' is actually less than 6 per cent. It should be emphasized that these are *average* operating characteristics and are not inconsistent with the larger rate of false negative diagnosis among young girls.

DISCUSSION

This study demonstrates that both age and sex affect the serum phosphorus level and must be taken into account in making diagnostic decisions. The proposed functions of serum inorganic phosphorus on age are an improvement over presently "generally accepted" values. There are, however, 4 criticisms of the proposed zones of normalcy which should be discussed.

1. *The normal values taken from the literature and supplemented by a large number of unpublished determinations from several investigators may not be based on a suitable normal population.* Although all investigators supplying data were dependable and used the same phosphorus method, each laboratory undoubtedly had made slight technical modifications. Each modification, however slight, increases the inter-laboratory variation, thus increasing the total variation in collated data. As implied by the discussion of laboratory variation under Materials and Methods, one cannot be certain whether a slight but significant difference between two presumably similar populations assessed by different laboratories represents a difference between the observers or between the observed. In a practical sense, the inflationary influence of pooling data from several laboratories is beneficial if the discriminant is to be generally applicable, because the inter-laboratory variation will have been taken into account *a priori*.

2. *The normal values are not evenly distributed with respect to age.* It can be seen in Figures 1 and 2 that most of the normal values were obtained on sera from younger persons and that there were few determinations on persons older than 60 years. The concentration of observations in the early decades may have distorted the shapes of the curves for older persons to some extent. The over-all effect was probably to spread somewhat wider

the true zones of normalcy. Despite this bias, not a single elderly person with postrachitic deformities or transmitting hypophosphatemia to an affected child was classified as normal by this phosphorus discriminant.

3. *On individual patients the data with respect to time were cross-sectional rather than longitudinal.* This is another way of saying that there is no assurance that the present group of 6-year-old subjects represents correctly what the present 30-year-old subjects were twenty-four years ago. This criticism is applicable to any investigation limited to a single point in time which purports to describe the effect of age on a biologic parameter.

4. *Abrupt changes in the concentration of serum inorganic phosphorus which might have occurred within an individual subject immediately prior to a single test were not always counteracted by averaging multiple determinations obtained at different times.* This factor obviously tends to widen the zone of normalcy. However, there is some advantage in a slightly exaggerated zone of normalcy. The criticism is more applicable to the normal than to the abnormal population, because several determinations were made on almost all of the abnormal subjects and averaged.

The lower limit of the zone of normalcy has been shown to be an efficient discriminant for correctly diagnosing hypophosphatemia occurring in families with "uncomplicated" vitamin D resistant rickets of the sex-linked variety. We have demonstrated that there is never a greater chance than about 0.5 per cent of scoring a normal person as abnormal (α' in Table 2). The discriminant makes more errors, however, in incorrectly scoring abnormal persons as normal. At first it appeared that the average false negative diagnostic rate was as high as 6–9 per cent. Later, the true operating characteristics of the discriminant were actually found to be much better. It appears in fact that there is probably less than a 5 per cent chance *on the average* that a man whose serum phosphorus level is in the normal range will be abnormal, and less than a 3 per cent chance *on the average* that a woman whose serum phosphorus level is in the normal range will be abnormal (β' in Table 2).

Unfortunately, the discriminant is not as efficient in correctly diagnosing affected young females. The false negative diagnostic rate with girls may reach 15 per cent in the first decade (Fig. 4), and we have observed a deficiency of affected females recently which seems to have originated in this fashion (3). Fortunately the discriminant becomes more efficient with age and can be used with high confidence by the third decade. We have not yet observed a false negative diagnosis in the transmitting mother of an affected child.

It should be emphasized that the operating characteristics of the phosphorus discriminant *do not necessarily apply to hypophosphatemic states other than those associated with "uncomplicated" vitamin D resistant rickets.*

The delineation of the operating characteristics in other hypophosphatemic states must await compilation and analysis of abnormal data in a manner similar to the one employed in this study. This is especially true for such conditions as parathyroid hyperactivity, a disorder involving patients in all stages of development. In these instances it might be wiser to restrict the lower confidence limit of the normal population to 95 per cent or even 90 per cent and accept a higher rate of false positive diagnosis in order to detect a larger proportion of the early cases. It is for use in such circumstances that the lower 95 per cent confidence limit has been placed in Figures 1 and 2.

REFERENCES

1. WINTERS, R. W.; GRAHAM, J. B.; WILLIAMS, T. F.; MCFALLS, V. W., and BURNETT, C. H.: A genetic study of familial hypophosphatemia and vitamin D resistant rickets, *Tr. A. Am. Physicians* **70:** 234, 1957.
2. WINTERS, R. W.; GRAHAM, J. B.; WILLIAMS, T. F.; MCFALLS, V. W., and BURNETT, C. H.: A genetic study of familial hypophosphatemia and vitamin D resistant rickets with a review of the literature, *Medicine* **37:** 97, 1958.
3. GRAHAM, J. B.; MCFALLS, V. W., and WINTERS, R. W.: Familial hypophosphatemia with vitamin D resistant rickets. II. Three additional kindreds of the sex-linked dominant type with a genetic analysis of four such families, *Am. J. Human Genetics* **11:** 311, 1959.
4. HOWLAND, J., and KRAMER, B.: Calcium and phosphorus in the serum in relation to rickets, *Am. J. Dis. Child.* **22:** 105, 1921.
5. TISDALL, F. F., and HARRIS, R. I.: Calcium and phosphorus metabolism in patients with fractures, *J.A.M.A.* **79:** 884, 1922.
6. STEARNS, G., and WARWEG, E.: Studies of phosphorus of blood. I. The partition of phosphorus in whole blood and serum, the serum calcium and plasma phosphatase from birth to maturity, *J. Biol. Chem.* **102:** 749, 1933.
7. BULLOCK, J. K.: The physiologic variations in the inorganic blood phosphorus content at the different age periods, *Am. J. Dis. Child.* **40:** 725, 1930.
8. HAMILTON, J. B.; BUNCH, L., and HIRSCHMAN, A.: Serum inorganic phosphorus levels in males and females at progressive ages, with concomitant measurements of urinary ketosteroids and androgens in men, *J. Clin. Endocrinol. & Metab.* **16:** 463, 1956.
9. FRANK, H. A., and CARR, M. H.: "Normal" serum electrolytes with a note on seasonal and menstrual variation, *J. Lab. & Clin. Med.* **49:** 246, 1957.
10. BOGDONOFF, M. D.; SHOCK, N. W., and NICHOLS, M. P.: Calcium, phosphorus, nitrogen, and potassium balances in the aged male, *J. Gerontol.* **8:** 272, 1953.
11. BRYAN, A. H. (Univ. of North Carolina): Personal communication.
12. STEARNS, G. (State Univ. of Iowa): Personal communication.
13. HAMILTON, J. B. (State Univ. of New York, Downstate Med. Center): Personal communication.
14. FISKE, C. H., and SUBBAROW, Y.: The colorimetric determination of phosphorus, *J. Biol. Chem.* **66:** 375, 1925.
15. TODD, W. R.; CHUINARD, E. G., and WOOD, M. T.: Blood calcium and phosphorus in the newborn, *Am. J. Dis. Child.* **57:** 1278, 1939.

16. BARNES, D. J., and MUNKS, B.: Serum phosphatase, calcium, and phosphorus values in infancy, *Proc. Soc. Exper. Biol. & Med.* **44**: 327, 1940.
17. GITTELMAN, I. F., and PINCUS, J. B.: Influence of diet on the occurrence of hyperphosphatemia and hypocalcemia in the newborn infant, *Pediatrics* **8**: 778, 1954.
18. GRAHAM, G. C.; BARNESS, L. A., and GYÖRGY, P.: Serum calcium and inorganic phosphate in the newborn infant, and their relation to different feedings, *J. Pediat.* **42**: 401, 1953.
19. BRUCK, E., and WEINTRAUB, D. H.: Serum calcium and phosphorus in premature and full-term infants. A longitudinal study in the first three weeks of life, *A.M.A. Am. J. Dis. Child.* **90**: 653, 1955.
20. VON SYDOW, G.: A study of the development of rickets in premature infants, *Acta paediat.* **33**: Suppl. 2, 1946.

APPENDIX

The problem is to derive the extent of the effect upon the operating characteristics of a diagnostic index caused by basing the calculations for it upon data which have been subjected to original clinical mis-classification errors of α_0 and β_0.

Using the same symbols as in the main body of the paper, consider a population of N persons, p of whom are normal, and r of whom are abnormal. If a perfect device for classifying all of them existed, there would be pN normal persons and rN abnormal ones. If the population were classified by a purely clinical system subject to the errors α_0 and β_0 (false positive and false negative rates, respectively), the expected number of persons in the various categories would be as shown in Table 3.

Thus, instead of finding rN cases of the disease by means of the perfect classification, the substitution of a clinical scheme subject to error results in an expected number of abnormal persons equal to $\alpha_0 pN+(1-\beta_0)rN$. Similarly, instead of pN normals, the expected number of presumed normals is equal to $\beta_0 rN+(1-\alpha_0)pN$.

Suppose that a new laboratory diagnostic test is developed which supplements the purely clinical scheme. Also suppose that this new index has as operating characteristics in this classification scheme, false positive and false negative rates equal to α and β. If this new test were based upon data obtained in turn from a perfect classification, there would be αpN false positive and βrN false negative diagnoses. On the other hand, if this new index were based upon the expected number of abnormal and normal sub-

TABLE 3

Imperfect clinical classification	Perfect classification		
	Normal	Abnormal	Total
Normal	Correctly classified $(1-\alpha_0)pN$	False negative diagnoses $\beta_0 rN$	$\beta_0 rN+(1-\alpha_0)pN$ $=[(p-\alpha_0 p+\beta_0 r)N]$
Abnormal	False positive diagnoses $\alpha_0 pN$	Correctly classified $(1-\beta_0)rN$	$\alpha_0 pN+(1-\beta_0)rN$ $=[(r+\alpha_0 p-\beta_0 r)N]$
Total	Truly normal persons pN	Truly abnormal persons rN	N

jects from the imperfect clinical classification, the following numbers of persons would be misclassified according to the new diagnostic test: False positives $=\alpha(p-\alpha_0 p+\beta_0 r)N$; and false negatives $=\beta(r+\alpha_0 p-\beta_0 r)N$. However, $\alpha pN \neq \alpha(p-\alpha_0 p+\beta_0 r)N$ and $\beta rN \neq \beta(r+\alpha_0 p-\beta_0 r)N$, because a perfectly classified population is used in the first instance and a misclassified population in the second. However, there are two unknown quantities α' and β', the effective rates of misclassification starting with a misclassified population, which compensate for the original misclassification when substituted for α and β in the expression for a misclassified population. This substitution then allows the foregoing listed quantities to be equated.

When this is done:

$$\alpha'(p - \alpha_0 p + \beta_0 r)N = \alpha pN,$$

and

$$\beta'(r + \alpha_0 p - \beta_0 r)N = \beta rN,$$

and it becomes possible to solve for α' and β', thus determining the effective rates of misclassification:

$$\alpha' = \alpha\left(\frac{p}{p - \alpha_0 p + \beta_0 r}\right)$$

$$\beta' = \beta\left(\frac{r}{r + \alpha_0 p - \beta_0 r}\right)$$

Some limitation exists in this formulation. If the values for α_0 and β_0 are too large (both equal to 0.5 or greater), then the validity of the diagnostic index itself must be questioned because it will have been based upon misleading basic data.

JCEM

FURTHER EVIDENCE FOR A THYROID ACTIVATOR IN HYPERTHYROIDISM*

J. M. McKENZIE, M.D.†

The Ziskind Research Laboratories, New England Center Hospital, and the Department of Medicine, Tufts University School of Medicine, Boston, Massachusetts

ABSTRACT

In mice fed desiccated thyroid (U.S.P.) the intravenous injection of sera from patients with hyperthyroidism caused an increased thyroidal uptake of I^{131} if given twelve hours before injection of the radioisotope. This was distinct from the effect of standard thyrotropin which produced a maximal increase if injected four hours before the I^{131}. Activation of the mouse thyroid gland by the injected sera was indicated also by an increase in the level of the protein-bound I^{127} of the plasma and by histologic changes in the thyroid.

THE intravenous injection of thyrotropin will cause an increase in the circulating radioactivity of mice previously given radioiodine and fed thyroid U.S.P. On this basis a sensitive assay for thyrotropin in serum was developed (1), and the increase in I^{131} was found to be maximal within two to three hours when pituitary thyrotropin or serum from persons with hypothyroidism was injected. Confirming the work of Adams and Purves (2) and Adams (3), a delayed response maximal at eight to twelve hours was found following the injection of certain sera which, in the main, came from patients with hyperthyroidism (4). Clearly, however, this delayed increase of I^{131} in blood was not proof of thyroid activation; theoretically at least, it could have been caused by the breakdown of thyroid tissue and the escape of I^{131} into the circulation. Proof of thyroid activation has now been obtained by other than the original criterion.

METHODS

The techniques for assaying thyrotropin and detecting the delayed response have been described (4). When the uptake of I^{131} by the thyroid of the mouse was to be measured, the animal was killed by chloroform inhalation twelve hours after the intraperitoneal injection of I^{131}, the thyroid gland and trachea were removed, and the radioactivity measured in a well-type scintillation counter. When required, as much blood as possible (0.6 ml. to 1.1 ml.) was obtained by percutaneous cardiac puncture of the

Received July 9, 1959.

* A portion of this work was reported at the 72nd Annual Meeting of the Association of American Physicians, Atlantic City, N. J., May 1959.

† Present address: Department of Medicine, Royal Victoria Hospital, Montreal, P. Q., Canada.

TABLE 1. SUMMARY OF CLINICAL AND LABORATORY DATA*

Patient, age and sex	Diagnosis and comment	Serum PBI (μg./100 ml.)	Thyroidal 24-hr. uptake of I^{131} (% dose)	Goiter, estimated weight (Gm.)	Exophthalmos	Pretibial myxedema
F.W., 35 yrs., female	Hyperthyroidism, receiving propylthiouracil	2.4	68	50	0	0
E.D., 59 yrs., female	Hyperthyroidism	15.1	80.1	30	0	0
E.G., 17 yrs., female	Hyperthyroidism	15.4	112	60	0	0
A.M., 60 yrs., male	Hyperthyroidism; propylthiouracil stopped 2 days previously	6.9	82	40	+	+
R.M., 49 yrs., female	Hypothyroidism	2.4	13.6	30	0	0

* The data were obtained at the time the blood was taken from the patients for assay and the other procedures detailed in the text.

anesthetized mouse. The concentration of protein-bound iodine (PBI) in the plasma was measured by the method of Zak and associates (5). Thyroid glands for histologic study, attached to the trachea, were fixed in formol-saline, sectioned, and stained with hematoxylin and eosin.

Throughout all of these experiments the mice were fed thyroid U.S.P. in a concentration of 0.066 per cent in the diet (Ken-L-Biskit) in order to achieve thorough suppression of endogenous thyrotropin (1).

Sera from 4 patients with hyperthyroidism and 1 with hypothyroidism were obtained and stored for not more than one week at 4° C. before use in these studies. Brief clinical and laboratory data on these patients are shown in Table 1. The 4 patients with hyperthyroidism were from a group of 50 whose sera had been tested for a delayed response in the assay for thyrotropin. In 42 of these patients the sera produced a delayed response; it is hoped to publish details of this group at a later date.

RESULTS

In Table 2 are shown the results of the assays of serum for thyrotropin in these 5 patients. The type of response obtained with the sera of patients

TABLE 2. ASSAY OF SERA FOR THYROTROPIN

Patient	Diagnosis	Mean response (%)	
		at 2 hrs.	at 9 hrs.
F.W.	Hyperthyroidism	130	152
E.D.	Hyperthyroidism	103	202
E.G.	Hyperthyroidism	162	295
A.M.	Hyperthyroidism	118	289
R.M.	Hypothyroidism	274	158

Serum was assayed as previously described (4). In every case 0.5 ml. of serum was given intravenously to each of 5 mice that had previously received an injection of I^{131}. The response was measured by the radioactivity in the blood of the test animals, 2 hours and 9 hours later, and expressed as a percentage of the radioactivity before the injection of serum. Interpretation of the responses is made in the text.

F.W., E.D., E.G. and A.M., greater at nine hours than at two hours, was the delayed response previously described with sera from similar thyrotoxic patients (4). The response to 0.5 ml. of the serum from Patient R.M. (hypothyroidism) was maximal at two hours and was similar to that obtained with injection of 0.15 milliunit (mu.) of U.S.P. Thyrotropin Reference Standard; thus R.M.'s serum was estimated to contain approximately 0.3 mu. of thyrotropin per ml.

Intravenous injection, every twelve hours, of 0.5 ml. of serum from Patient E.G. or A.M. maintained for at least four days a high concentration of circulating I^{131} in the mice. The results obtained with the serum of Patient E.G. are shown in Figure 1.

The serum of Patient E.G. was used to determine the effect of repeated

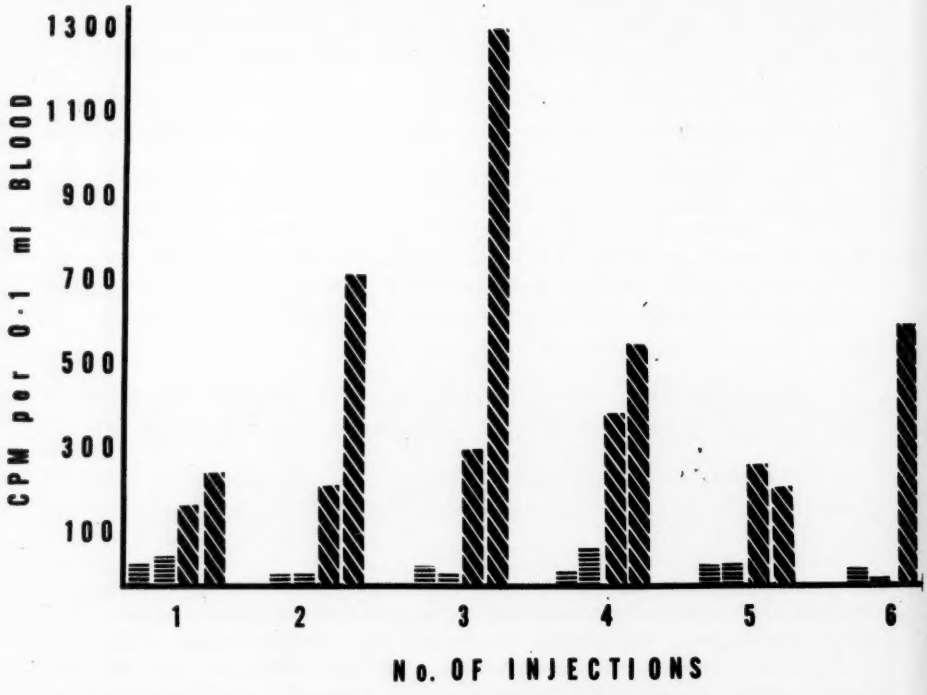


FIG. 1. The effect of repeated injections of serum from a hyperthyroid patient on the I^{131} content (counts per minute) of the blood of mice. The vertical bars represent the values in individual mice which were given intravenous injections of serum from Patient E.G. (oblique stripes) or physiologic sodium chloride (horizontal stripes) every twelve hours. The volume of each injection was 0.5 ml. Blood for the counting of contained radioactivity was obtained twelve hours after the last injection; 0.1 ml. was plated on an aluminum planchette, and counted in a gas-flow counter with a thin end-window.

injections on the level of protein-bound I^{127} in the plasma of the mice. The results are shown in Table 3. Mice were given intravenous injections of 0.5 ml. of serum at intervals of twelve hours; other mice similarly were given physiologic sodium chloride solution, and all were exsanguinated twelve hours after the last of 4 such injections. The protein-bound iodine level in the plasma of the mice receiving the serum was significantly greater than that in the control animals ($P < 0.01$). To test the possibility that the protein-bound iodine measured in the plasma of the mice might have been partly that in the serum which had been injected, sodium *l*-thyroxine was

TABLE 3. EFFECT OF INTRAVENOUS INJECTIONS OF SERUM FROM A HYPERTHYROID PATIENT ON THE PLASMA PROTEIN-BOUND IODINE LEVEL OF MICE

Thyroid in diet	Treatment*	No. of mice	PBI (μg./100 ml. plasma)	
			Mean	Standard Deviation
0.066%	0.5 ml. serum q. 12 hrs. for 2 days	4	7.075**	0.637
0.066%	0.5 ml. 0.9% NaCl sol. q. 12 hrs. for 2 days	4	3.35**	1.125
0.066%	2.7 μg. *l*-thyroxine daily for 5 days	6	3.0	—
Nil	Nil	6 (pooled)	2.0	—

* Intravenous injections of serum or of 0.9% NaCl were made every twelve hours and the mice were exsanguinated twelve hours after the last injection.

Sodium *l*-thyroxine was injected in 0.2 ml. of 0.9% NaCl solution subcutaneously and the animals were exsanguinated twenty-four hours after the last injection.

** "*t*" test of significance of difference of means, $P < 0.01$.

given to other mice in a total dose of 13.5 μg. per mouse over five days. There was no change in the concentration of protein-bound iodine during this treatment.

The effect of intravenous injection of these sera on the thyroidal uptake of I^{131} was studied, varying the time interval between the administration of serum and the subsequent injection of I^{131}. Control groups of mice received injections of 0.9 per cent sodium chloride solution. Sera from Patients F.W., E.D., E.G. and A.M. caused an increase in the thyroidal uptake of radioiodine and this increase was maximal when I^{131} was injected twelve hours after the intravenous injection of serum; serum from Patient R.M. (hypothyroidism) had no effect on the uptake when similarly tested. Representative results, obtained with sera from Patients A.M., E.G. and R.M. are shown in Table 4.

The length of time before which an intravenous injection of U.S.P.

TABLE 4. STIMULATION OF THYROIDAL UPTAKE OF I^{131} IN THE MOUSE BY INTRAVENOUS INJECTION OF HUMAN SERUM*

		Hours between injection of serum or salt solution and injection of I^{131}						P
		0	4	8	12	16	24	
Thyroidal I^{131} uptake (% dose)								
After serum of Patient A.M.	Mean	1.49	1.76	1.37	*4.23*		2.3	
	S.D.†	0.33	0.64	0.18	0.84		0.78	<0.001
After 0.9% sol. of NaCl	Mean	0.94					1.05	
	S.D.	0.35					0.23	
After serum of Patient E.G.	Mean		5.63		*11.18*	8.01		
	S.D.		3.2		2.06	4.34		<0.001
After 0.9% sol. of NaCl	Mean				3.73			
	S.D.				1.98			
After serum of Patient R.M.	Mean		3.25		*3.7*			
	S.D.		1.16		0.78			Not significant
After 0.9% sol. of NaCl	Mean		1.84		2.95			
	S.D.		0.61		1.23			

* All groups consisted of 5 or 6 mice.

I^{131} was given by intraperitoneal injection 0–24 hours after serum or 0.9% solution of NaCl had been given as a single intravenous injection in a volume of 0.5 ml.; the thyroidal uptake of I^{131} was measured after a further twelve hours.

The P value (Student's "*t*" test) in the last column indicates the significance of the difference of the greatest mean uptake (italicized) from the corresponding control figures.

† Standard Deviation.

Thyrotropin Reference Standard exerted maximal effect on the thyroidal uptake of I^{131} was determined. Six groups of 5 mice each received 10 mu. of thyrotropin, intravenously; radioiodine was given by intraperitoneal injection 0–12 hours later, and the thyroidal uptake of I^{131} was measured after a further twelve hours. The maximal effect occurred with an interval of four hours between the administrations of thyrotropin and of I^{131}; a similar but no greater effect was found when the interval was eight or twelve hours (Table 5). The mean thyroidal uptake of I^{131} by the control mice in the studies listed in Tables 4 and 5 ranged from 0.94 to 5.3 per cent. These differences are accounted for by variations in the length of time (two to four days) during which the animals were treated with thyroid

TABLE 5. TIME OF MAXIMAL EFFECT OF INTRAVENOUS THYROTROPIN ON THYROIDAL UPTAKE OF I^{131}*

Hours between injection of thyrotropin or 0.9% sol. of NaCl and injection of I^{131} →		0	1	3	4	8	12
Thyroidal I^{131} uptake (% dose) after thyrotropin	Mean	7.79	12.46	16.1**	29.6**	27.65	29.86
	S.D.†	3.45	8.6	8.1	8.8	8.9	9.8
Thyroidal I^{131} uptake (% dose) after 0.9% sol. of NaCl →	Mean	5.3					
	S.D.	2.4					

* All groups consisted of 5 mice.

I^{131} was given by intraperitoneal injection 0–12 hours after administration of the thyrotropin (10 mu. U.S.P. in 0.2 ml. of 0.9% solution of NaCl) or 0.9% solution of NaCl (0.2 ml.), and the thyroidal uptake of I^{131} was measured after a further twelve hours.

** "*t*" test of significance of difference of means at two hours and four hours; P, 0.05–0.02.

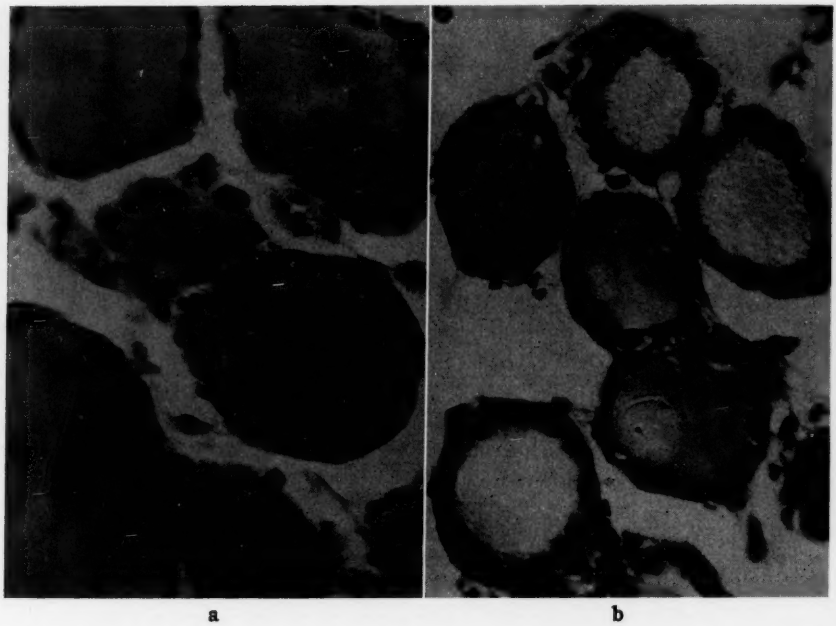
† Standard Deviation.

before the uptake was determined. All control groups of mice, however, were treated with thyroid for the same period of time as the corresponding serum-injection groups.

Observations were also made on the changes in histologic structure of the thyroid glands of the mice following repeated injections of the sera from Patients E.G. and A.M. (0.5 ml. intravenously every twelve hours). Physiologic sodium chloride solution was again given to other mice for control studies. For each serum being tested, 2 control and 2 test animals were killed every twelve hours, starting twelve hours after the first injections had been given. The thyroids were examined histologically. As a consequence of the suppression of endogenous thyrotropin by the desiccated thyroid in the diet, the thyroid glands of the control mice uniformly had acini with flat epithelium and densely staining colloid. No significant change was observed in the glands of the animals receiving injections of serum until from seven to nine injections had been given, when there was an increase in acinar cell height, reduced intensity of staining of the colloid, and formation of vacuoles. An example of these changes is shown in Figure 2.

DISCUSSION

It is clear that there was some factor in the serum of the patients with hyperthyroidism which activated the thyroid glands of the mice receiving



a b

FIG. 2. The effect of injections of serum from a patient with hyperthyroidism (E.G.) on the histologic structure of the thyroid of the mouse. **a.** Thyroid of control mouse after injection of 0.9% NaCl. **b.** Thyroid of a mouse after injection of serum. (Hematoxylin and eosin, ×595.) Both mice were fed thyroid U.S.P. (see "Methods") and injections were given in a volume of 0.5 ml. every twelve hours for a total of 7 injections. Each mouse was killed twelve hours after the last injection.

injections of this serum. This was borne out by radiobiologic, chemical and histologic data. It would seem safe to conclude, therefore, that the delayed response shown in these animals by the increase in circulating I^{131} nine hours after the injection of serum was indicative of thyroid activation. Presumably the similar delayed response noted in previous assays of sera (2–4) had the same significance. For the last thirty years there has been doubt about the presence of thyrotropin in the blood in hyperthyroidism, and contradictory evidence resulted from bioassays (6). Adams's work (3) with its confirmation (4) and the present report establish that there is a circulating thyroid-activating principle in at least some persons with hyperthyroidism. However, there is an unexplained qualitative difference between this principle and thyrotropin from pituitary extracts or from the blood in hypothyroidism, *viz*, a delay in effect as judged by the thyroidal discharge of I^{131}. Similarly, its effect on the thyroidal uptake of I^{131} has been shown qualitatively to differ from that of standard thyrotropin. This dif-

ference might possibly be due to an abnormal metabolism of thyrotropin in hyperthyroidism; it is even conceivable that the activity in the blood of these patients is associated with a substance distinct from thyrotropin.

It has been shown by various workers that thyrotropin causes discharge of iodine-containing compounds from the thyroid gland before any effect on the uptake of I^{131} is demonstrable (7, 8). The maximal effect of an intravenous injection of thyrotropin on the thyroidal discharge of I^{131}-labeled substances into the blood of the mouse has been shown to occur after two hours (1). The finding reported here that thyrotropin similarly administered to the mouse exerted a maximal effect on the thyroidal uptake of I^{131} after a delay of four hours is thus in keeping with the data of these previous reports. A maximal increase in the I^{131} level of the blood of mice receiving injections of serum from patients with hyperthyroidism was originally reported to occur in about twelve hours (4). Experience with the assay since that time has shown that the maximal effect occurs about eight or nine hours after injection. It is therefore noteworthy that the maximal stimulation of thyroidal I^{131} uptake produced by these sera should occur even later, with twelve hours elapsing between the administration of serum and the subsequent injection of I^{131}.

Adams (3) suggested that this thyroid activator in the serum in hyperthyroidism was the "exophthalmos-producing substance" of Dobyns and Wilson (9). Although this has not been excluded, no confirmation was obtained in the present study. The serum of Patient A.M., the only subject with exophthalmos (and pretibial myxedema), elicited a response quantitatively very similar to that obtained with the serum of the other patients with hyperthyroidism, particularly Patient E.G. This young girl exhibited no abnormal eye features other than moderate retraction of the eyelids. Studies in progress appear to confirm the lack of correlation between the degree of the delayed response obtained in the assay of the serum and the degree of the patient's exophthalmos.

Acknowledgments

I am grateful to Dr. E. B. Astwood for much helpful criticism.

This study was supported by funds from the American Cyanamid Company and the U. S. Public Health Service (Grant A-612).

REFERENCES

1. McKenzie, J. M.: The bioassay of thyrotropin in serum, *Endocrinology* **63**: 372 (Sept.) 1958.
2. Adams, D. D., and Purves, H. D.: Abnormal responses in the assay of thyrotropin, *Proc. Univ. Otago Med. School* **34**: 11, 1956.
3. Adam , D. D.: The presence of an abnormal thyroid-stimulating hormone in the serum of some thyrotoxic patients, *J. Clin. Endocrinol. & Metab.* **18**: 699 (July) 1958.

4. McKenzie, J. M.: Delayed thyroid response to serum from thyrotoxic patients, *Endocrinology* **62:** 865 (June) 1958.
5. Zak, B.; Willard, H. H.; Myers, G. B., and Boyle, A.: Chloric acid method for determination of protein-bound iodine, *Analyt. Chem.* **24:** 1345 (Aug.) 1952.
6. McKenzie, J. M.: The assay of thyrotropin in urine and blood, *in* Progress in Clinical Endocrinology, ed. by E. B. Astwood, New York, Grune & Stratton, 1960.
7. Keating, F. R., Jr.; Rawson, R. W.; Peacock, W., and Evans, R. D.: The collection and loss of radioactive iodine compared with the anatomic changes induced in the thyroid of the chick by the injection of thyrotropic hormone, *Endocrinology* **36:** 137 (Feb.) 1945.
8. Wahlberg, P.: The effect of thyrotropic hormone on thyroid function, *Acta endocrinol.* Suppl. 23, 1955.
9. Dobyns, B. M., and Wilson, L. A.: An exophthalmos-producing substance in the serum of patients suffering from progressive exophthalmos, *J. Clin. Endocrinol. & Metab.* **14:** 1393 (Nov.) 1954.

DIAGNOSTIC LIMITS FOR THYROIDAL RADIOIODINE UPTAKE RATES*

T. H. ODDIE, D.Sc., I. D. THOMAS, M.B., B.S., F. F. RUNDLE, M.D., J. MYHILL, B.Sc.† AND B. CATT, B.Sc.

Unit of Clinical Investigation, Royal North Shore Hospital of Sydney, Crows Nest, N.S.W., Australia

ABSTRACT

The thyroidal I^{131} uptake rate was measured in 1,116 Australian subjects whose thyroidal status was established at the same time. The logarithm of the uptake rate factor for various functional groups was found to be normally distributed. Diagnostic borderlines are described for premenopausal and postmenopausal females with and without goiter, and for males with and without goiter. The I^{131} uptake rate was found to decrease progressively with age in male subjects. In females it remained constant until the age of the menopause, following which a progressive decrease was seen. In 76 per cent of subjects unaffected by prior medication a clear diagnosis was established by a single thyroidal I^{131} uptake test.

INTRODUCTION

MEASUREMENT of the iodine uptake rate describes only one facet of thyroidal function. The rate of discharge of thyroid hormone from the gland may be quite dissociated from the rates of iodine assimilation and hormone formation. Since the pattern of dissociation of these parameters is of diagnostic value, it is clear that it is not justifiable to use terms such as "hyperthyroid" and "hypothyroid" applied to a single thyroid function without excluding from consideration those conditions in which dissociation may occur, namely, recent thyroidectomy, thyroiditis, goitrous cretinism and recent medication with thyroid-affecting drugs.

In the application of the method (1) of uptake measurement used in this Unit it soon became evident that the diagnostic ranges described in the original work (derived predominantly from British and North American populations) were not applicable to a normal Australian population. The objects of the present work are firstly to define the diagnostic ranges for the local population, and secondly to describe the observed differences between subjects of different sexes and age groups; it is probable that these latter variations would be found in other populations.

Received August 17, 1959.

* Work partly carried out in the Joan Bonamy Research Laboratory and supported by a generous grant from an anonymous donor.

† Supported by a grant from the Australian Atomic Energy Commission.

METHODS

Subjects

The 1,116 subjects of this study were volunteers and patients seen in a hospital clinic and in private practice during the last twenty-seven months. All were of European extraction and were resident in New South Wales, a state in which many country districts are recognized as being mildly endemic goiter areas. In analyzing the results, however, there was no separation of groups on a domiciliary basis.[1]

The study included only subjects whose thyroidal radioiodine uptake rate was unaffected by prior medication, who were free from inflammatory or infiltrative lesions of the thyroid, who showed no clinical evidence of abnormal hormone synthesis, whose thyroid gland was impalpable or could be wholly palpated in the neck and in whom a clear diagnosis could be established on review of all data.

Reasons for excluding cases were: 1) cancer of the thyroid, 2) thyroiditis, 3) intrathoracic goiter, 4) goitrous cretinism, 5) thyroidectomy less than six months previously, 6) antithyroid drugs less than two years previously, 7) other goitrogenic drugs less than one year previously, 8) iodide less than one year previously, 9) x-ray opaque oils and dyes less than two years previously, and 10) desiccated thyroid (or analog) less than two months previously.

The remaining group of 1,116 cases was made up of 45 hypothyroid, 963 euthyroid and 108 hyperthyroid subjects.

Determination of thyroidal status

The thyroidal status of each subject was determined in retrospect after review of all available data. These comprised in all patients careful clinical evaluation, including the response to treatment, and thyroidal radioiodine uptake tests; in many patients supplementary tests were performed.

Clinically hyperthyroid patients with very high I^{131} uptake rates, euthyroid subjects with uptake rates near the middle of the normal range, or hypothyroid patients with no thyroidal uptake of I^{131} were admitted to the study without requiring further tests. Often, however, further tests were performed in such cases.

All subjects with a moderately elevated I^{131} uptake rate were subjected to a thyroxine suppression test, or to a chemical estimation of the level of serum protein-bound iodine, or to both.

All patients with a low uptake rate (provided it was appreciably above zero) underwent estimation of the serum protein-bound iodine level or received an injection of pituitary thyrotropic hormone followed by a further radioiodine uptake test.

The radioiodine uptake rate factor, k_1 minute $^{-1}$, was determined in all cases with intravenous tracer doses of 5 to 10 μc. of I^{131}. Counts were made over the thyroid gland shortly after injection and about one hour later, using a collimated scintillation counter with a 2-inch diameter crystal; urine collected during this time was also assayed. These observations enabled corrections to be made for renal excretion and body and neck tissue background. A full description of these methods has already been published (1). The rate, k_1, represents the fraction of the iodide pool cleared per minute by the thyroid gland. The thyroidal uptake of I^{131} is measured at any suitable time from thirty minutes to several hours after the injection of the tracer dose, but k_1 is always calculated to represent conditions when the iodide has diffused into the iodide space for thirty minutes after injection.

[1] However, only about 15 per cent of the subjects came from rural areas; most were residents of Sydney and other coastal metropolitan areas.

Chemical estimations of the serum protein-bound iodine level were made by the method of Zak *et al.* (2) as modified by Leffler (3). Diagnostic ranges for the test as used in this study were (in μg. per 100 ml):

less than 3.5—hypothyroid
3.5 to 3.9—no decision
4.0 to 8.0—euthyroid
8.1 to 8.5—no decision
more than 8.5—hyperthyroid.

When thyroxine suppression tests were performed, k_1 was measured initially, and again after the patient had received from 0.4 mg. to 0.7 mg. of *l*-thyroxine sodium daily in divided dosage orally for from ten to twenty-one days; the higher dosage was used when the smaller produced neither a significant fall in the I^{131} uptake rate nor clinical evidence of an increased metabolic rate. In hyperthyroid patients k_1 showed occasionally a very small reduction but more often a rise in the second test, whereas in euthyroid subjects k_1 on the second occasion had fallen either to zero or to a very low value. To assist in separation when the response was doubtful, the total hormone flow was estimated by a method (4) described previously.

Size of goiter

An estimate of the size of the thyroid gland was made by palpation of the neck in each patient. Sizes were graded as:

0—Normal; only the thyroid isthmus palpable.
1—Both lobes of the thyroid discernible but not notably enlarged.
2—Moderate enlargement of the thyroid.
3—Large goiter.
4—Gross enlargement of the thyroid.

Patients suspected of having intrathoracic goiter were examined by a special radiographic technique (5). If the suspicion was confirmed, such patients were excluded from this analysis.

RESULTS

Statistical distribution of k_1 values

A preliminary examination of the total pooled group of k_1 values showed that k_1 itself had a markedly skew distribution. The distribution of log k_1 was found to be bimodal with a symmetrical main peak and a smaller peak at higher log k_1 values.

More detailed analysis of homogeneous groups of patients confirmed this result, namely, that k_1 followed a log-normal distribution with reasonable accuracy. For example, χ^2 tests yielded the following values of P, the probability that the departure of the observed distribution of log k_1 from the assumed normal distribution was fortuitous:

For euthyroid females aged from 13 to 45, without goiter, $P = 0.015$.
For euthyroid females aged from 13 to 45, with goiter, $P = 0.25$.
For all hyperthyroid patients of either sex, and any age, with or without goiter, $P = 0.75$.

Figure 1 shows a histogram for the 108 hyperthyroid patients, with the superimposed ideal curve.

Thus for further statistical treatment it was necessary to convert the uptake rates to the logarithmic form, for which a convenient parameter is

$$y = 3 + \log k_1 \qquad [1]$$

because k_1 itself is of the order of 0.003 minute^{-1}.

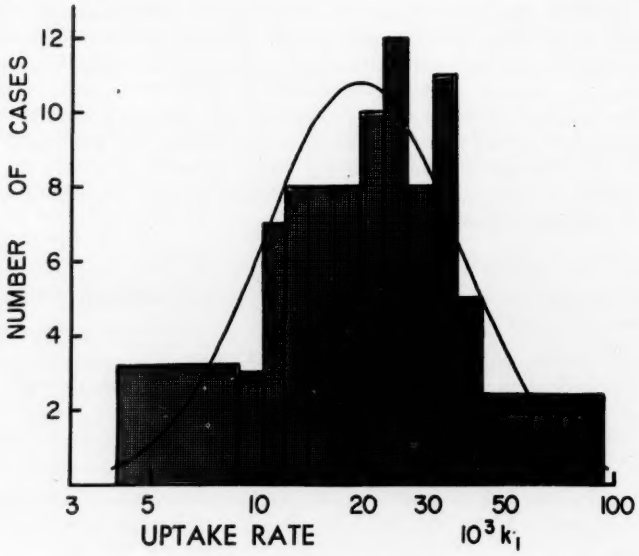


FIG. 1. Long-normal distribution of k_1 for 108 proved thyrotoxic patients of both sexes and all age groups.

Mean values and pooling of results for euthyroid subjects

In Table 1 are listed the mean values of the logarithmic parameter y for different classes of euthyroid subjects, together with the standard deviations of these means. Results were pooled for patients with goiters of all sizes (other than 0) because there were insufficient numbers in each group to yield significant differences between the mean values of y.

The tabulated results demonstrated clearly significant differences between the male and female groups, and between comparable groups with and without goiter. The values of y for males showed a general decrease with increasing age. In contrast, the values for females remained practically constant up to about 45 years of age, and then decreased with increasing age.

TABLE 1. MEAN VALUES OF UPTAKE PARAMETER y FOR 963 PROVED EUTHYROID SUBJECTS

($y = 3 + \log k_1$; errors listed are standard deviations of the mean values)

Age (yrs.)	Female				Male			
	Without goiter		With goiter		Without goiter		With goiter	
	No.	Mean y	No.	Mean y	No.	Mean y	No.	Mean y
13–20	24	.450 ± .057	37	.704 ± .044	8	.488 ± .068	7	.721 ± .185
21–25	23	.489 ± .032	53	.614 ± .035	23	.480 ± .025	4	.475 ± .179
26–30	29	.416 ± .046	67	.678 ± .033	11	.496 ± .056	6	.500 ± .123
31–35	45	.517 ± .028	73	.642 ± .029	11	.404 ± .061	5	.470 ± .111
36–40	59	.530 ± .027	78	.614 ± .029	3	.383 ± .120	6	.367 ± .046
41–45	40	.508 ± .038	55	.601 ± .028	7	.336 ± .173	6	.583 ± .080
46–50	42	.393 ± .035	40	.450 ± .040	11	.350 ± .058	1	.750
51–55	21	.331 ± .042	33	.404 ± .046	11	.277 ± .080	9	.394 ± .050
56–60	21	.407 ± .043	16	.512 ± .051	6	.150 ± .093	2	.250
61–65	14	.264 ± .066	11	.459 ± .051	6	.300 ± .042	0	
66–70	9	.294 ± .037	9	.261 ± .121	1	.650	0	
71–75	5	.390 ± .092	6	.500 ± .049	0		1	.250
76+	5	.190 ± .169	3	.383 ± .030	0		0	
Total	337		481		98		47	

Accordingly the data for males were examined in two groups only (with and without goiter), but for females in four groups (age 13 to 45, and age 46 upwards, each with and without goiter). Within each group, calculations were made to test a linear relationship between y and age (x years), with the results shown in Table 2. In these calculations the assumed equation is

$$y = a + bx \qquad [2]$$

The best linear fit for the observed data is represented by equation 2, with the various calculated values of a and b listed in Table 2. However, at the 5 per cent level ($P = 0.05$ or less) only the regression coefficients, b, for the two male groups differ significantly from zero.

In all cases in which comparable groups of subjects were compared at their mean age, those with goiter had a significantly higher y than those without goiter. The difference was about constant for the three pairs of groups.

The groups of females aged 13 to 45, with and without goiter had, re-

TABLE 2. TEST OF LINEAR RELATIONSHIP BETWEEN PARAMETER y AND AGE x OF EUTHYROID SUBJECTS

Quantity	Female				Male	
	Without goiter		With goiter			
	Age 13–45	Age 46 up	Age 13–45	Age 46 up	Without goiter	With goiter
Number of cases	220	117	363	118	98	47
Correlation coefficient r	0.1181	0.1776	0.0954	0.0548	0.3664	0.3070
P*	0.079	0.052	0.069	0.555	<0.001	0.031
a	0.3951	0.6060	0.7325	0.5249	0.6060	0.7321
10^3b	3.059	−4.494	−2.976	−1.649	−5.691	−6.527
S.D. of 10^3b	1.743	2.316	1.634	2.790	1.474	3.022

* From a t test for significance, P is the probability that the correlation between x and y is only fortuitous.

spectively, negative and positive coefficients, b, neither of which was significant. Accordingly, for further pooling of data, b was taken as zero in these cases.

On physiologic grounds, females past 45 years of age might be expected to show a gradual decline of thyroid function, such as occurs in males. With this expectation in mind, the two negative values of b for the older groups of females were combined with the b values for males, to provide a weighted mean

$$10^3b = -4.944 \pm 1.063 \qquad [3]$$

TABLE 3. MEAN DIFFERENCES BETWEEN y VALUES FOR GOITROUS AND NONGOITROUS SUBJECTS

(errors tabulated are standard deviations)

Sex	Age range (yrs.)	Mean value of a		
		With goiter, a_g	Without goiter, a_o	Difference, $a_g - a_o$
F	13–45	0.6387 ± 0.0132	0.4950 ± 0.0145	0.1437 ± 0.0196
F	46 up	0.7085 ± 0.0230	0.6313 ± 0.0201	0.0772 ± 0.0306
M	All	0.6735 ± 0.0415	0.5781 ± 0.0215	0.0954 ± 0.0468
Weighted mean difference				0.1213 ± 0.0156

TABLE 4. FINAL EQUATIONS FOR POOLED MEAN y VALUES FOR EUTHYROID SUBJECTS

Sex	Age range	Goiter	Equation	Standard deviation for individual patient
F	13–45	0	$y=0.5025$	0.2146
F	13–45	+	$y=0.6238$	0.2532
F	46+	0	$y=0.6162-4.944\times10^{-3}x$	0.2178
F	46+	+	$y=0.7375-4.944\times10^{-3}x$	0.2507
M	All	0	$y=0.5732-4.944\times10^{-3}x$	0.2131
M	All	+	$y=0.6945-4.944\times10^{-3}x$	0.2861

With this value of b, the mean values of the constant, a, in equation 1 were recalculated, yielding the differences between goitrous (a_g) and nongoitrous (a_o) groups shown in Table 3.

It was evident that (a_g-a_o) differed significantly from zero for each pair ($P<.001$ for females 13 to 45, $P=.012$ for females 46 and upwards, and $P=.041$ for males).

However, the variation of (a_g-a_o) between the three sets was not significant; therefore the weighted mean difference was selected for further use. Thus the combined results were represented as in Table 4.

Figures 2 and 3 show these combined linear relations, together with the mean points from Table 1.

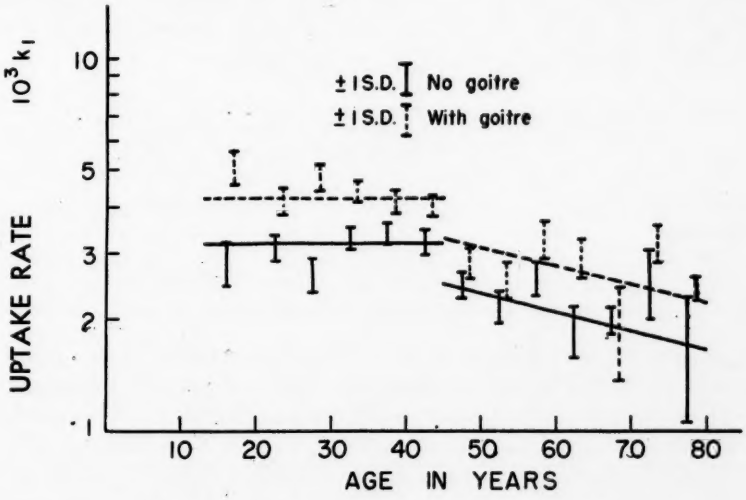


FIG. 2. Mean thyroidal I^{131} uptake rates for euthyroid females unaffected by prior medication.

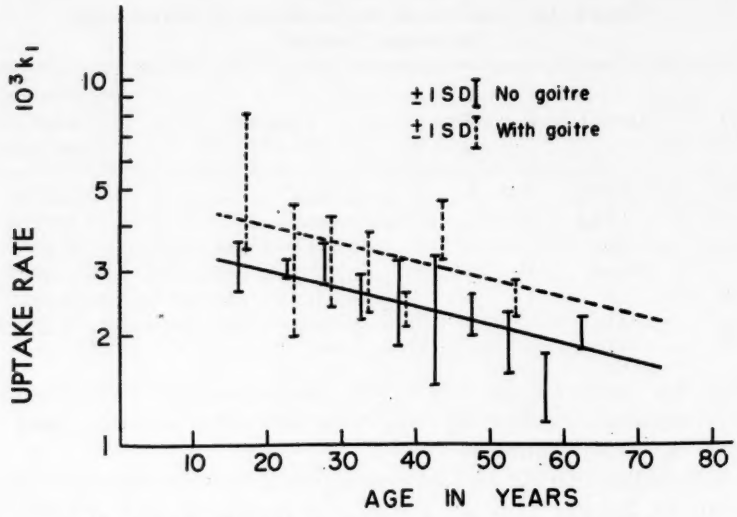


FIG. 3. Mean thyroidal I^{131} uptake rates for euthyroid males unaffected by prior medication.

Mean values for hyperthyroid subjects

The logarithmic parameter y was normally distributed for hyperthyroid as well as for euthyroid subjects (Fig. 1). The mean results for 108 proved hyperthyroid patients, listed in Table 5, showed no significant differences between the age groups. There was an insufficient number of cases to warrant separate grouping by sex or by size of the goiter.

TABLE 5. MEAN VALUES OF UPTAKE PARAMETER y FOR 108 PROVED HYPERTHYROID PATIENTS

Age range (yrs.)	No. of cases	Mean y	Standard deviation	
			Of mean	Of individual case
0–12	3	1.485	0.139	0.241
13–25	8	1.264	0.082	0.233
26–40	41	1.284	0.047	0.300
41–55	40	1.291	0.041	0.260
56 up	16	1.270	0.069	0.274
All	108	1.289	0.026	0.272

Results for hypothyroid cases

Many hypothyroid subjects had a k_1 value of zero or nearly zero, so that use of the logarithmic parameter y was not convenient. However, information was required on how high a k_1 or y may be found in hypothyroidism. Of the 45 proved cases studied, 21 had zero uptakes and the remaining 24 had k_1 ranging up to 0.61×10^{-3}; the highest 11 of these are plotted in Figure 4, which shows the differences by which the observed y differed from that calculated from the mean euthyroid figures for the appropriate age, sex, and goiter size. The least observed difference in y on this basis was 0.626.

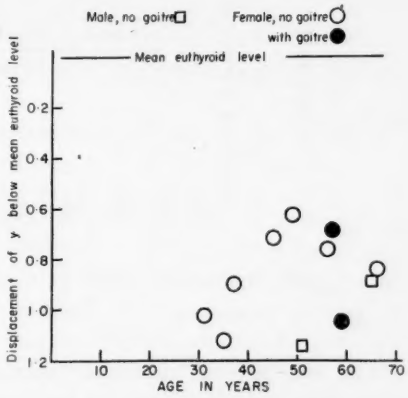


FIG. 4. Distribution of highest results obtained in patients with hypothyroidism.

CHOICE OF DIAGNOSTIC LIMITS

With the data accumulated on the statistical spread of the uptake parameter y, an informed choice of diagnostic limits may be made.

In this Unit the limits for y at present used are:

Border A—The appropriate mean euthyroid y from Table 4 minus 3.09 standard deviations.
Border B—The mean euthyroid y minus 0.500.
Border C—The mean hyperthyroid y minus 2.05 standard deviations.
Border D—The mean euthyroid y plus 3.09 standard deviations.

Figure 5 illustrates these borders (converted to k_1) for females without goiter; similar charts or tables are readily prepared for other classes of patients. With the foregoing limits, the "clearly hypothyroid" region below A is expected to include about 96 per cent of all hypothyroid cases and 0.1 per cent of all euthyroid cases; the latter will be wrongly diagnosed by a single test result.

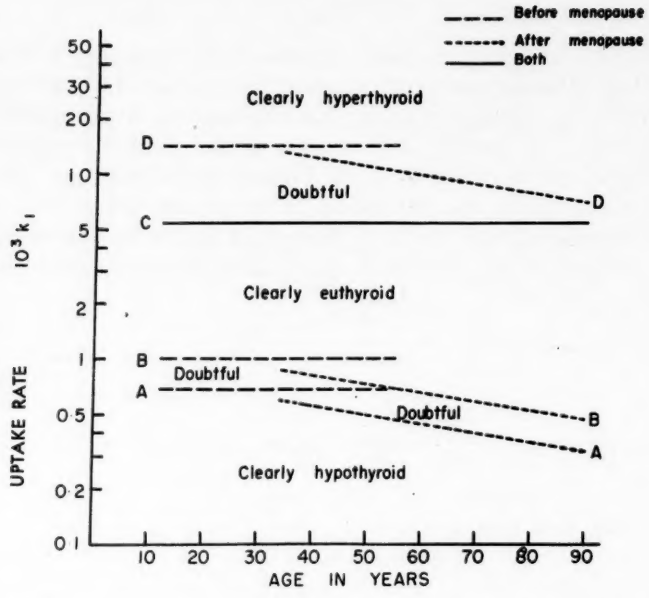


FIG. 5. Diagnostic limits for females without goiter and unaffected by prior medication.

The overlapping "doubtful" region between A and B will include about 4 per cent of the hypothyroid group and about 1.8 per cent of the euthyroid group.

The "clearly euthyroid" zone between B and C will include 78 per cent of the euthyroid cases and about 2 per cent of the hyperthyroid patients, the latter being incorrectly diagnosed by this one test.

The upper overlapping "doubtful" region between C and D will include about 20 per cent of the euthyroid cases and 50 per cent of the hyperthyroid patients. And finally the "clearly hyperthyroid" region above D will include 0.1 per cent of the euthyroid cases, wrongly diagnosed by the one test, and 48 per cent of the hyperthyroid patients.

DISCUSSION

The bimodal distribution of the pooled results deserves comment. The smaller peak at the higher values of uptake rate is caused by the hyperthyroid patients. It has been pointed out by Pickering and Peart (6), in relation to arterial blood pressure, that the measured value of a body function is not necessarily to be considered abnormal simply because it departs

from the average; when the spread of all observed values in health and disease fits a unimodal distribution, then it is improbable that the function being studied is primarily culpable for associated disease. Since the hyperthyroid patients accounted for the secondary peak in our observations it might be concluded that hyperthyroidism constitutes a disease entity, as distinct from representing one extreme of normal function.

The average normal values for the thyroidal I^{131} uptake rate obtained in this study are greater than the normal values described previously (1) for a different population, by a factor of about 3.6. There is no evidence that the output of hormone by the thyroid gland in Australia is greater than that in Britain or the United States of America, and it appears likely that this higher uptake rate reflects a state of relative iodine deficiency of the thyroid, secondary either to a smaller iodide pool associated with iodine-deficient food intake or possibly to the chronic ingestion of naturally occurring (8) goitrogens.

It is of interest to note the progressive fall with age of the values obtained for males. The recognition that aging alone is associated with a gradual fall in at least one thyroid function is consistent with well-known histologic features (7). The phenomenon should be borne in mind when evaluating the long-term effects of any form of treatment for thyrotoxicosis.

Although the thyroidal I^{131} uptake rates in males show a progressive decline with age, this decline is not seen in females until after the age of about 45. It seems likely (though here unproven) that ovarian activity accounts for the maintenance of thyroidal function at a constant level in the premenopausal years. It would seem reasonable therefore to use the extrapolated "over 45" diagnostic boundaries for a younger woman who had reached the menopause, whereas a woman over 45 who was still menstruating regularly should be judged by the "under 45" boundaries.

With the borderlines described, 5 zones are delineated. In 3 of these zones a clear diagnosis is claimed, but in the remaining 2 the diagnosis remains doubtful on the basis of a single uptake test and must be elucidated by further investigation. Over-all, about 76 per cent of cases fall into a zone of clear diagnosis by one test, and 24 per cent require some form of further investigation. On the basis of one thyroidal I^{131} uptake test alone, 1 in 1000 euthyroid patients will be wrongly classified as hyperthyroid and 1 in 1000 as hypothyroid, and 2 per cent of hyperthyroid patients will be classified as euthyroid. In practice, such patients will usually be subjected to other investigations when there is an inconsistency between the clinical diagnosis and the uptake value.

It is emphasized that the observations detailed in this paper are valid only in the absence of prior thyroid-affecting medication.

Acknowledgment

The authors are indebted to Dr. F. Radcliff and Mrs. E. Hall, B.Sc., of the Kolling Institute of Medical Research, Royal North Shore Hospital, for the chemical determination of the serum protein-bound iodine level in many of the patients studied.

REFERENCES

1. Oddie, T. H.; Meschan, I., and Wortham, J. T.: Thyroid function assay with radioiodine. I. Physical basis of study of early phase of iodine metabolism and iodine uptake; II. Routine calculation of thyroidal and renal rate constants; III. Clinical test results and diagnostic limits for rate constants, *J. Clin. Invest.* **34**: 95, 106 and 1044, 1955.
2. Zak, B.; Willard, H.; Myers, G. B., and Boyle, A. J.: Chloric acid method for determination of protein bound iodine, *Analyt. Chem.* **24**: 1345, 1952.
3. Leffler, H. H.: The determination of protein bound iodine, *Am. J. Clin. Path.* **24**: 483, 1954.
4. Oddie, T. H., and Thomas, I. D.: Radio-iodine studies of the equilibrium turnover of thyroxine in the thyroid gland, *in* Symposium on the Peaceful Uses of Atomic Energy in Australia. Sydney, Australia, Atomic Energy Commission, 1958, p. 669.
5. Rundle, F. F.; de Lambert, R. M., and Epps, R. G.: Cervicothoracic tumors: a technical aid to their roentgenologic localization, *Am. J. Roentgenol.* **81**: 316, 1959.
6. Pickering, G. W., and Peart, W. S.: Hypertension, *in* Biochemical Disorders in Human Disease, ed. by R. H. S. Thompson, and E. J. King. London, Churchill, 1957, p. 155.
7. Thomas, F.: L'histophysiologie de la glande thyroïde humaine à la lumière de tests morphologiques nouveaux, *Arch. de biol.*, Paris **45**: 189, 1934.
8. Clements, F. W., and Wishart, J. W.: A thyroid-blocking agent in the etiology of endemic goiter, *Metabolism* **5**: 623, 1956.

THE EFFECTS OF SEVERAL THYROXINE ANALOGUES UPON THE THYROIDAL I^{131} UPTAKE IN MAN*

STANLEY SLATER, M.D., MARTIN PERLMUTTER, M.D., JULIO PAZ-CARRANZA, M.D. AND MARVIN NUMEROFF, M.A.

The Medical Services of the Maimonides Hospital of Brooklyn, and the Department of Medicine, State University of New York, College of Medicine, Brooklyn, New York

ABSTRACT

The 24-hour thyroidal radioiodine uptake was determined in a group of euthyroid and hyperthyroid human subjects before and after the administration of one of several analogues of thyroxine. The analogues used were 3,5,3′-*l*-triiodothyronine (3′-T_3); 3,3′,5′-*dl*-triiodothyronine (5′-T_3); 3,5,3′-triiodothyroacetic acid (Triac); 3,5,3′,5′-tetraiodothyroacetic acid (Tetrac), and 3,5,3′-triiodothyropropionic acid (Trip). The minimal dose of each analogue which was effective in inhibiting thyroidal I^{131} uptake in the euthyroid subjects was ascertained. If 3′-T_3 is assigned a potency of 100, the potencies of the other analogues as determined in this manner were: 5′-T_3, <1; Triac, 2.5; Tetrac, 5; and Trip, 2.5. These projected potencies were compared with those derived by other investigators using different techniques in several species of animals in addition to man. In the present series, with the dosages used, these analogues had no effect upon thyroidal I^{131} uptake in any of the hyperthyroid subjects tested.

A NUMBER of analogues of thyroxine have been used in attempts to elucidate further the physiology and pathology of the thyroid gland, and to try to identify compounds with a therapeutic effect greater than, or different from that of desiccated thyroid or *l*-thyroxine (1). Varying techniques employing several species of animals have been used in these studies. The present report describes the effect of several of these compounds upon the thyroidal uptake of radioiodine in man.

METHOD

The patients were taken at random from those upon whom 24-hour radioiodine (I^{131}) uptake tests had been performed in the isotope laboratory of the Maimonides Hospital. After the initial (control) uptake had been determined, each patient was provided with a seven-day supply of one of the thyroxine analogues, to be taken in a single dose once daily. On the seventh day of ingestion of the drug, a background count was performed to determine residual radioactivity and a larger, second tracer dose of radioiodine

Received June 24, 1959.

* Abstract No. 123, Program of the Fortieth Meeting of The Endocrine Society, San Francisco, California, June 19–21, 1958.

was administered. Determination of the 24-hour uptake over the thyroid gland was repeated the following day. The radioiodine was always administered orally. In patients with a nodular goiter, directional counting was performed at the time of the initial uptake determination in order to ascertain whether the nodules were "hot."

Evaluation of the thyroid function of each patient was made without reference to the result of the analogue suppression test. The final decision as to the state of thyroid function was based on the clinical findings, the chemical serum protein-bound iodine (PBI) level and the response to therapy. No untoward effects were detected from the analogues used. The normal range for thyroidal 24-hour iodine uptake in this laboratory is 15–50 per cent of the dose. The degree of decline in the uptake was calculated from the formula:

$$\% \text{ decline in uptake} = \frac{\text{initial uptake} - \text{final uptake}}{\text{initial uptake}} \times 100.$$

In a previous study, it was found that desiccated thyroid and 3,5,3′-*l*-triiodothyronine (3′-T_3) induce a decline in uptake amounting to 30 per cent or more in euthyroid persons (2). This criterion was applied to the results of the present study to decide if a significant reduction was induced by the analogue tested. Repeat uptakes in control subjects receiving no analogue varied by less than 10 per cent.

RESULTS

The results obtained in the euthyroid subjects are presented in Table 1. The analogues tested and the daily dose administered are listed in the first two columns. The mean initial control thyroidal I^{131} uptake is listed for each drug and dosage in the third column, except for 3,3′,5′-*dl*-triiodothyronine (5′-T_3) which was taken by too small a number of subjects[1]. The fourth column shows the mean percentage decline in I^{131} uptake induced by the various doses of the several analogues. The last two columns show the number of euthyroid subjects whose uptake failed to be significantly depressed (*i.e.*, less than 30 per cent) and the total number of euthyroid subjects tested with the same dose of a particular analogue. In some of the euthyroid subjects whose uptakes were not altered by the analogue tested, the thyroid gland contained a "hot"[2] nodule; in others, the uptake was not influenced by a subsequent course of 3′-T_3 in a dosage of 0.1 mg. daily. Appropriate notation is made of these subjects, since lack of decline in these instances probably was due to certain characteristics of the thyroid gland rather than to ineffectiveness of the compound tested.

The initial pre-analogue mean uptakes of I^{131} in the several groups tested were of the same order of magnitude. Observed differences in the degree of decline were therefore not likely due to differences in the subjects tested.

It can be seen that a daily dose of 0.035 mg. of 3′-T_3 was uniformly[3] ef-

[1] The initial uptakes were 35, 66 and 30 per cent in those receiving 1 mg. of this analogue daily and 29, 39, 41 and 34 per cent in those receiving the 15 mg. daily.

[2] Uptake of I^{131} more than that of normal thyroid tissue.

[3] This uniformity of suppression was fortuitous, since 2 subjects tested with other analogues subsequently failed to experience suppression of uptake after 0.1 mg. of 3′-T_3.

TABLE 1. EFFECT OF THYROXINE ANALOGUES ON THYROIDAL UPTAKE OF I^{131} IN EUTHYROID SUBJECTS

Analogue*	Daily dose (mg.)	Pre-analogue thyroidal I^{131} uptake (% dose)	Decline in uptake after analogue (% and S.D.)	No. of euthyroid subjects with decline of less than 30%	Total No. of euthyroid subjects
3′-T_3	0.035	42.5±2.4	69.5±3.1	0	27
5′-T_3	1.0		0	3	3
	15.0		47.0**	1	4
Trip	0.5	42.8±2.4	36.6±6.3	4	14
	1.0	40.0±2.1	61.6±3.9	3†	34
Tetrac	0.25	40.9±2.9	32.4±6.8	8	15
	0.50	37.2±3.1	56.9±4.7	1	15
	0.75	45.7±2.7	63.5±6.0	2‡	15
	1.0	42.6±2.4	59.3±4.7	4§	27
Triac	0.2	38.8±2.3	28.8±5.8	7	11
	0.4	43.9±3.9	35.0±6.2	4	7
	0.6	43.2±3.5	34.4±7.4	5	11
	0.8	46.0±2.7	49.5±7.0	3	15
	1.0	40.2±2.1	59.6±4.3	4	34

* 3′-T_3 = 3,5,3′-triiodothyronine.
5′-T_3 = 3,3′,5′-triiodothyronine.
Trip = 3,5,3′-triiodothyropropionic acid.
Tetrac = 3,5,3′,5′-tetraiodothyroacetic acid.
Triac = 3,5,3′-triiodothyroacetic acid.

** The mean for the 3 subjects in whom uptake was suppressed; values for the other analogues include all the subjects tested.

† Includes 1 subject with a "hot" nodule and 1 subject whose uptake was not suppressed by 0.1 mg. of 3′-T_3.

‡ Includes 1 subject with a "hot" nodule in whom the uptake was not suppressed by 3′-T_3.

§ Includes 3 subjects with "hot" nodules.

fective in depressing thyroidal I^{131} uptakes in the subjects tested, and the percentage reduction of uptake was also higher than that obtained with any of the other analogues. The 5′ analogue of T_3, on the other hand, was ineffective in depressing the uptake in 3 subjects given a dose of 1 mg. daily. When a daily dose of 15 mg. of 5′-T_3 was administered, a reduction was observed in 3 of 4 subjects tested; the average decline induced in these 3 subjects was only 47 per cent. Thus, the 5′ analogue of T_3 is much less effective than the 3′ analogue in causing suppression of the thyroidal uptake of radioiodine.

A dosage of 0.5 mg. daily of 3,5,3′-triiodothyropropionic acid (Trip) was relatively ineffective as judged by the mean percentage decline in the uptake and by the proportion of subjects in whom no reduction was obtained. In only 3 of the 34 subjects was there no decline in uptake during administration of 1 mg. of Trip daily; furthermore, no reduction was obtained in 1 of these 3 with a subsequent course of 0.1 mg. of 3′-T_3 daily and another had a "hot" nodule. If these qualifying factors are considered, it can be seen that 1 mg. of Trip daily almost uniformly caused depression of the uptake, and that the magnitude of the decline approximated that caused by 0.035 mg. of 3′-T_3 daily.

A daily dose of 0.25 mg. of 3,5,3′,5′-tetraiodothyroacetic acid (Tetrac) failed to inhibit uptake in a significant number of the subjects tested, and the mean decline induced was relatively small. Considering the qualifications discussed in the preceding paragraph, it can be seen that inhibition was almost uniformly obtained with daily dosages of 0.5, 0.75 and 1.0 mg. of Tetrac. Furthermore, the magnitude of the decline was approximately the same for each of these dosages.

As the daily dose of 3,5,3′-triiodothyroacetic acid (Triac) was increased from 0.2 mg. by 0.2-mg. increments, there was a gradual decrease in the proportion of subjects whose uptakes were not inhibited; even at the 1.0-mg. level, however, the results were not quite as good as with the other analogues. The magnitude of the decline in thyroidal uptake rose progressively as the daily dose of Triac was increased, until at the 1.0-mg. level the degree of inhibition was comparable with the maximum degree obtained with the other analogues.

The various analogues in the dosages used failed to inhibit the thyroidal I^{131} uptake in any of the hyperthyroid subjects. These data are presented in Table 2.

TABLE 2. EFFECT OF THYROXINE ANALOGUES UPON THYROIDAL UPTAKE OF I^{131} IN HYPERTHYROID SUBJECTS

Analogue	Daily dose (mg.)	No. of subjects tested	No. of subjects with decline in uptake of 30% or more
Triac	0.2	1	0
	0.8	1	0
	1.0	6	0
Trip	1.0	5	0
Tetrac	0.25	1	0
	0.50	1	0
	1.0	1	0

DISCUSSION

Our use of relatively large doses of the thyroxine analogues raises the question as to whether the results noted were due to a thyroxine-like action of the analogue itself or to inorganic iodine which may have been released from the analogue *in vivo*. One milligram of each of the analogues used contains approximately 0.66 mg. of iodine. We have not administered stable iodine in this dosage to ascertain its effect. Starr and Liebhold-Schueck (3) found that 0.42 mg. of iodine daily did not influence the thyroidal uptake but a daily dose of 2.1 mg. did. Similarly, Greer (4) found that the administration of 0.38 mg. of iodine daily (as potassium iodide) had minimal, if any, effect upon the uptake. Based upon these data, it would seem that in the subjects receiving 0.5 mg. of Tetrac daily it is reasonably certain that the results obtained were not due to inorganic iodine. Since the results with this dosage were similar to those with larger doses of the analogues, it is not necessary to invoke a different mechanism to explain the data. Corroborative evidence may be found in determinations of the serum PBI level carried out in many cases before and after administration of the analogue for one week (5). With Trip and Triac there was little change in the PBI level. In subjects receiving Tetrac, there was usually a rise in the concentration of PBI for each dosage used. In some patients, however, there was a significant inhibition of thyroidal uptake with no change in the PBI level, whereas in others there was a marked rise in the PBI level with no demonstrable effect on the uptake. If the decline in thyroidal uptake was due to release of inorganic iodine, one would expect a better correlation between the rise of serum PBI concentration and the fall of thyroidal uptake.

The same dosage levels of the several analogues did not interfere with the thyroidal I^{131} uptake in any of the hyperthyroid subjects nor in some of the euthyroid subjects with "hot" nodules. Some of the hyperthyroid subjects received Tetrac and the elevations of the serum PBI level were of the same magnitude as in the euthyroid persons whose thyroidal I^{131} uptakes were inhibited.

In comparing the effects of the several analogues upon the thyroidal uptake of I^{131}, the absorption of the test material from the gastro-intestinal tract must be considered. Acute experiments were performed in which the concentration of serum PBI was measured serially after the oral administration of a single dose of each analogue. The limited data available revealed a significant rise in the PBI level within a few hours after the ingestion of each analogue tested, except 5′-T_3. This result suggests that 5′-T_3 may be poorly, if at all, absorbed. It is possible that the apparently weak inhibitory action of 5′-T_3 on the thyroidal uptake is due to poor absorption rather than the lack of potency. At present there is no reason to suspect that the other analogues are not adequately absorbed.

TABLE 3. ASSAYS OF THYROXINE ANALOGUES

Type of assay	Species tested	Relative potency of analogue					
		T_4*	3′-T_3	5′-T_3	Triac	Tetrac	Trip
Goiter prevention (6, 7, 8, 9, 11)	Rat	10–20	100	1	10	10	3–20
Metamorphosis (6, 8, 10, 11)	Amphibian (tadpole)	10–20	100	0–1	340–480	20–200	80–6000
Inhibition of thyroidal I^{131} uptake (7)	Rat				0		
Clinical laboratory effects (12–16)	Hypothyroid humans	20–30	100		1.3–<7	0.6–1.2	
Depression of serum cholesterol level (13)	Euthyroid humans		100		4		
Inhibition of thyroidal I^{131} uptake (3, 17)	Euthyroid humans	2	100	<1	2.5	5	2.5

* Thyroxine.

In Table 3 is presented a compilation of the relative potencies of the analogues tested, as determined by various techniques. The data for a given type of assay were derived from the findings of one or more authors and recalculated in the following manner: When studies with 3′-T_3 were available as a standard with which to compare the activities of the other compounds, 3′-T_3 was arbitrarily assigned a potency of 100. When thyroxine (T_4) rather than 3′-T_3 had been used in a given experiment, T_4 was arbitrarily assigned a value of 20 since it was usually one fifth as potent as 3′-T_3 when compared in the same study. The other analogues were then assigned a potency which depended upon the relation of their minimally effective dose to that of 3′-T_3 or T_4.

The activities of T_4, 3′-T_3 and 5′-T_3, as estimated from their effectiveness in preventing the development of a goiter in rats fed a goitrogen and from their effect on tadpole metamorphosis, are in good agreement. Triac, Tetrac and Trip, on the other hand, are much more active than 3′-T_3 in causing tadpoles to metamorphose, but are considerably less active than 3′-T_3 in preventing development of goiter.

Courrier *et al.* (7) studied the inhibition of thyroidal radioiodine uptake in the rat. We could not arrive at numerical quantitation of his data, but 3′T_3 seemed to be more potent in this respect than did T_4, whereas Triac seemed to have no effect.

Many studies of thyroxine analogues in man have been performed by noting the clinical and laboratory effects in hypothyroid subjects, and the effect on the serum cholesterol level in euthyroid subjects. The results indicate a relative potency for Triac and Tetrac in the range of 1 to 5. This is considerably less than that obtained in other species of animals using different types of assays.

Some studies have been performed in man in which the ability of various dosages of thyroxine analogues to inhibit the thyroidal collection of radioiodine has been noted. Comparison of the minimal effective daily dosages of the various analogues, provides another form of assay. In our present study, we did not ascertain the minimal effective daily dose of 3′-T_3. Starr and Liebhold-Schueck (3) proposed that this dosage might be 0.0088 mg. but subsequent studies of McConahey and Owen (17) indicate that 0.025 mg. is the minimal daily amount needed to elicit a significant, reproducible decline in the iodine uptake. If the latter figure be arbitrarily accepted, then the potencies of Triac, Tetrac and Trip, as derived from the data of the present studies, are as noted in Table 3. These projected potencies are of the same order of magnitude as those obtained in man, using other techniques. The potency for T_4 as derived from the data of Starr *et al.*, however, indicate a potency considerably less than that derived by other techniques.

In comparing the potencies of the tri- and tetraiodinated derivatives of thyroacetic acid by the present technique, it would seem that the tetraiodinated derivative is more active than its triiodinated analogue. This provides another exception, therefore, to the general rule that trihalogenated thyronines are more active than their tetrahalogenated analogues (18). The apparent relative impotence of 5′-T_3 is compatible with the findings in other studies indicating its lack of effectiveness compared to 3′-T_3.

If further studies with other analogues confirm these results, they may provide a relatively simple assay for thyroxine analogues in man, and one which would not be dependent upon the availability of a supply of hypothyroid subjects.

Acknowledgment

The authors in indebted to Dr. Arthur Heming of Smith, Kline & French Laboratories for the supply of 3′-triiodothyronine and to Dr. Michael Barry of the Warner-Chilcott Laboratories for the other analogues used. They are most grateful to Miss Betty Pomerantz for her assistance in preparing the manuscript.

REFERENCES

1. Selenkow, H. A., and Asper, S. P., Jr.: Biological activity of compounds structurally related to thyroxine, *Physiol. Rev.* **35:** 426, 1955.
2. Perlmutter, M., and Slater, S.: Use of thyroid hormone to differentiate between hyperthyroidism and euthyroidism, *J.A.M.A.* **158:** 718, 1955.

3. STARR, P., and LIEBHOLD-SCHUECK, R.: Theory of thyroid hormone action, *A.M.A. Arch. Int. Med.* **92:** 880, 1953.
4. GREER, M.: The effect on endogenous thyroid activity of feeding desiccated thyroid to normal human subjects, *New England J. Med.* **244:** 385, 1951.
5. SLATER, S., and OTHERS: Unpublished data.
6. TOMITA, K., and LARDY, H.: Synthesis and biological activity of some triiodinated analogues of thyroxine, *J. Biol. Chem.* **219:** 595, 1956.
7. COURRIER, R.; ROCHE, J.; MICHEL, O.; MICHEL, R., and COLONGE, R. A.: Sur la régulation hormonale de la fixation thyroidienne des iodures et sur la pénétration des hormones thyroidiennes dans l'hypophyse, *Bull. Soc. Chim. biol.* Paris **38:** 1245, 1956.
8. MICHEL, R., and PITT-RIVERS, R.: The relative potencies of thyroxine and triiodothyronine analogues in vivo, *Biochim. et biophys. acta* **24:** 213, 1957.
9. PITT-RIVERS, R.: Physiological activity of the acetic acid analogues of some iodinated thyronines, *Lancet* **2:** 234, 1953.
10. MONEY, W. L.; MELTZER, R. I.; YOUNG, J., and RAWSON, R. W.: The effect of change in chemical structure of some thyroxine analogues on the metamorphosis of *Rana pipiens* tadpoles, *Endocrinology* **63:** 20, 1958.
11. ROCHE, J.; MICHEL, R.; TRUCHOT, R.; WOLF, W., and MICHEL, O.: Sur les activités biologiques des iodothyronines et de divers analogues structuraux des hormones thyroïdiennes, *Biochim. et biophys. acta* **20:** 337, 1956.
12. SELENKOW, H. A., and ASPER, S. P., JR.: The effectiveness of triiodothyronine or thyroxine administered orally in the treatment of myxedema, *J. Clin. Endocrinol. & Metab.* **15:** 285, 1955.
13. TROTTER, W. R.: Effect of triiodothyroacetic acid on blood cholesterol levels, *Lancet* **1:** 885, 1956.
14. RALL, J. E.; PEARSON, O. H.; LIPSETT, M. B., and RAWSON, R. W.: Metabolic effects in man of the acetic-acid analogues of thyroxine and triiodothyronine, *J. Clin. Endocrinol. & Metab.* **16:** 1299, 1956.
15. LERMAN, J., and PITT-RIVERS, R.: Physiologic activity of triiodo- and tetraiodothyroacetic acid in human myxedema, *J. Clin. Endocrinol. & Metab.* **16:** 1470, 1956.
16. GOOLDEN, A. W. G.: The physiologic activity of tetraiodothyroacetic acid, *Lancet* **1:** 890, 1956.
17. MCCONAHEY, W. M., and OWEN, C. A., JR.: Studies of the inhibitory effect of *l*-triiodothyronine on thyroidal uptake I^{131} in euthyroid persons and patients with exophthalmic goiter, *J. Clin. Endocrinol & Metab.* **16:** 1480, 1956.
18. MUSSETT, M. V., and PITT-RIVERS, R.: The physiologic activity of thyroxine and triiodothyronine analogues, *Metabolism* **6:** 18, 1957.

THE DIURETIC RESPONSE TO ADMINISTERED WATER IN PATIENTS WITH PRIMARY MYXEDEMA

KENNETH H. BLEIFER, CAPTAIN USAF (MC)*, JOSEPH L. BELSKY, M.D.†, LAWRENCE SAXON, M.D.‡ AND SOLOMON PAPPER, M.D.§

The Medical Service and the Research Laboratory, Boston Veterans Administration Hospital and the Departments of Medicine, Boston University School of Medicine and Tufts University School of Medicine, Boston, Massachusetts

ABSTRACT

The diuretic response to administered water was studied in 8 patients with typical, untreated primary myxedema. The maximal rate of urine flow was definitely reduced below the normal range (minimum, approximately 8 ml. per minute) in 2 of the 8 patients. In each of 5 patients re-studied following treatment with desiccated thyroid, including 3 whose pre-treatment peak flow of urine was greater than 8 ml. per minute, there was an increase in maximal diuretic response. The urine during periods of maximal flow after treatment was more dilute than before treatment in 4 of these patients. The rate of total solute or sodium excretion did not bear a consistent relationship to increased urine flow. Endogenous creatinine clearance increased significantly after treatment in all 5 patients. The modest limitation in water diuresis observed in primary myxedema may be attributed to a reduction in glomerular filtration rate. That the diuretic response to water is a useful test to differentiate primary myxedema from panhypopituitarism with secondary myxedema has been confirmed.

ALTHOUGH impairment in the ability to obtain a brisk diuresis following the administration of water has been clearly demonstrated in patients with hypopituitarism and secondary myxedema, the data with regard to primary myxedema are less well defined and somewhat contradictory. The present study demonstrates that modest impairment in

Received August 7, 1959.

* Formerly Resident in Medicine, Boston Veterans Administration Hospital and Teaching Fellow in Medicine, Tufts University School of Medicine.

Requests for reprints should be addressed to Dr. Solomon Papper, Medical College of Virginia Hospital, Richmond, Va.

† Staff Physician, Boston Veterans Administration Hospital, and Clinical Instructor in Medicine, Tufts University School of Medicine.

‡ Formerly United States Public Health Service Postdoctorate Research Fellow.

§ Formerly, Assistant Chief of Medicine, Boston Veterans Administration Hospital, and Associate Professor of Medicine, Tufts University School of Medicine. Presently, Associate Professor of Medicine, Medical College of Virginia, Richmond, Va.

water diuresis may occur in primary myxedema and may be attributed to a reduction in glomerular filtration rate.

MATERIALS AND METHODS

Eight patients with classic, untreated primary myxedema were studied. Isotonic hexose solution (20 ml. per Kg. of body weight) was administered intravenously within a one-hour period to each of these patients when they were normally hydrated and recumbent. Urine specimens were collected at approximately half-hour intervals until maximal rates of urine flow were achieved. On another occasion, 7 of the 8 patients were given a similar quantity of tap water orally. Five patients were studied again after treatment. Venous blood was obtained at the height of diuresis and at the end of the study. Urine and blood were analyzed for sodium, potassium, chloride, creatinine and total solute by methods previously described from this laboratory (1).

RESULTS

The results are summarized in Table 1.

Prior to treatment the maximal urine flow varied between 4.4 and 26.9 ml. per minute, with only 2 patients having flows less than 8.0 ml. per minute[1] when the water load was administered intravenously. There was little difference in peak flow when the water was given by mouth.

The urine during the periods of peak flow was between 63 and 142 milliosmolal, and the rate of solute excretion between 533 and 1810 microosmols per minute. The rate of sodium excretion varied from 53 to 541 μEq. per minute, and endogenous creatinine clearance from 83 to 214 ml. per minute.

Five patients were studied after thyroid therapy had been instituted and the clinical response observed. Maximal rates of urine flows were increased from 1.4 to 6.1 ml. per minute over pre-treatment values. In each instance this augmented diuresis was associated with an increase in endogenous creatinine clearance (12 to 56 ml. per minute). In 4 patients, the urine during the period of maximal flow was more dilute after treatment, and in 1 case the urine was more concentrated. In the latter instance, the increased diuresis after treatment was associated with a striking rise in the rate of excretion of sodium and total solute. In 2 other patients, increased diuresis was also associated with increased solute excretion; in 1 case the post-treatment rates of total solute and sodium excretion were lower than the pre-treatment values, and in 1 case there was virtually no difference between pre- and post-treatment rates of total solute excretion.

[1] In our laboratory, the range of maximal urine flow attained by normal persons under similar conditions is approximately 8 to 25 ml./min. The maximal diuretic response to the administration of water is largely determined by the glomerular filtration rate, the level of antidiuretic hormone activity (ADH), and the rate of solute excretion (2).

TABLE 1. EFFECT OF THYROID THERAPY ON WATER DIURESIS IN PRIMARY MYXEDEMA

Subject No.	Before treatment with thyroid										After treatment with thyroid									
	Urine flow (ml./min.)		Total solute (mOsm./Kg.)		Total solute (μOsm./min.)		Sodium (μEq./min.)		Creatinine clearance (ml./min.)		Urine flow (ml./min.)		Total solute (mOsm./Kg.)		Total solute (μOsm./min.)		Sodium (μEq./min.)		Creatinine clearance (ml./min.)	
	P.O.*	I.V.*	P.O.	I.V.	P.O.	I.V.	P.O.	I.V.	P.O.	I.V.	P.O.	I.V.	P.O.	I.V.	P.O.	I.V	P.O.	I.V.	P.O.	I.V.
1	8.1	8.1	130	83	1056	673	252	72	99	106	14.2		87		1238		341		117	
2	6.5	8.0	98	76	640	605	134	99	87	97	9.1	10.8	70	74	636	796	109	198	108	118
3	14.7	26.9	135	63	1810	1700	541	347	138	214	17.6		65		1000		210		194	
4		9.3		86		795		89		92		10.7		121		1289		475		112
5	4.4	4.9	110	136	533	675	13	95	107	100	8.7		96		836		141		119	
6	6.2	4.7	102	142	636	668	94	59	96	109										
7	8.6	12.3	96	110	832	1356	146	53	95											
8	8.9	9.1	97	85	870	720	205	127	118	83										

* Route of administration of the water load:
P.O. =by mouth.
I.V. =intravenously.

DISCUSSION

The diuretic response to administered water in patients with primary myxedema has been studied employing several experimental techniques, with results varyingly interpreted as normal (3–5) or abnormal (6, 7). In some instances, urine was not collected frequently enough to permit estimates of the maximal rate of flow following administration of water. In others, the water load may have been too small to assure maximal inhibition of antidiuretic hormone (ADH) secretion, thus making it difficult to compare the response of the hypothyroid patient with that of the normal subject. In addition, several of the studies describing "normal" response to administered water do not provide data allowing precise comparison of pre- and post-treatment maximal rates of urine flow. Finally, those studies demonstrating "abnormal" water diuresis do not completely define whether the mechanism of the limitation is related to decreased renal excretion of solute, enhanced ADH activity or reduction in glomerular filtration rate (GFR).

The present study demonstrates that modest impairment in water diuresis may occur in patients with primary myxedema. In several instances the peak urine flow prior to treatment may have been interpreted as within the broad range of normal; however, in each case treatment was followed by an increase in the maximal rate of urine flow attained. Although these data do not preclude the possibility of more severe limitation of water diuresis in primary myxedema, they confirm the fact that the capacity to excrete administered water is usually far greater in this condition than in hypopituitarism with secondary myxedema. In no case of panhypopituitarism in our series of approximately 20 patients has the maximal rate of urine flow exceeded 3.1 ml. per minute under comparable conditions. The diagnosis of panhypopituitarism in our patients was based on the clinical evidence of gonadal, thyroidal and adrenocortical insufficiency, as well as decreased urinary excretion of 17-ketosteroids and 11-hydroxycorticoids, decreased thyroidal uptake of radioactive iodine (I^{131}) and a reduced concentration of serum protein-bound iodine (PBI). In addition each patient had radiographic evidence of an intrasellar mass; and in each of several instances in which the determination was made, the urinary titer of follicle-stimulating hormone was reduced. Finally, clinical remission (including the diuretic response to administered water) required the use of cortisone replacement therapy. Primary myxedema was assumed to be a reasonable diagnosis in patients with typical symptoms who had a reduction in thyroidal I^{131} uptake and the serum level of PBI without an intrasellar lesion, and in whom clinical response was complete with the use of thyroid hormonal replacement therapy alone. In several patients in this category,

modest reduction in adrenocortical steroid excretion was observed. In several instances the response to thyroid-stimulating hormone (TSH) was also used as a differentiating test.

In attempting to define the mechanism of the impaired water diuresis observed in primary myxedema, the following considerations are pertinent. Since the urine during the period of peak flow was more dilute after treatment in 4 of the 5 patients, one cannot exclude the possibility that the pre-treatment limitation in water diuresis was at least in part related to enhanced ADH activity. This cannot be established with certainty in view of concomitant post-treatment changes in solute excretion and endogenous creatinine clearance. The fifth patient, whose urine was more concentrated during the period of peak flow after treatment than before treatment, had the smallest increment in diuretic response to administered water. This increase in flow was associated with a striking rise in solute excretion and an increase of 20 ml. per minute in creatinine clearance. However, 2 patients in the group restudied after treatment demonstrated increased water diuresis without augmentation in solute excretion. Consequently, a precise correlation between enhanced diuresis and altered ADH activity or rates of solute excretion is not possible. On the other hand, in each instance a significant increase in endogenous creatinine clearance was observed following treatment, suggesting the possibility that the pre-treatment limitation in water diuresis was determined largely by a reduction in GFR. Results of other studies, including a more precise measurement of glomerular filtration rate in myxedema, confirm this finding (8, 9). The mechanism of this reduction in patients with myxedema cannot be inferred from the present study; however, the observed reduction in cardiac output in this condition may well account for the changes in renal hemodynamics (10)

REFERENCES

1. Birchard, W. H.; Prout, T. E.; Williams, T. F., and Rosenbaum, J. D.: Diuretic responses to oral and intravenous water loads in patients with hepatic cirrhosis, *J. Lab. & Clin. Med.* **48**: 26, 1956.
2. Rosenbaum, J. D.; Nelson, W. P., III; Strauss, M. B.; Davis, R. K., and Ross meisl, E. C.: Variation in the diuretic response to ingested water related to the renal excretion of solutes, *J. Clin. Invest.* **32**: 394, 1953.
3. Oleesky, S.: Water diuresis in primary myxedema, *Lancet* **1**: 256, 1955.
4. Levy, M. S.; Power, M. H., and Kepler, E. J.: The specificity of the "water test" as a diagnostic procedure in Addison's disease, *J. Clin. Endocrinol.* **6**: 607, 1946.
5. Moses, A. M.; Gabrilove, J. L., and Soffer, L. J.: Simplified water loading test in hypoadrenocorticism and hypothyroidism, *J. Clin. Endocrinol. & Metab.* **18**: 1413, 1958.
6. Hubble, D.: Endocrine relations, *Lancet* **1**: 1, 1955.
7. Crispell, K. R.; Parson, W., and Sprinkle, P.: A cortisone-resistant abnormality

in the diuretic response to ingested water in primary myxedema, *J. Clin. Endocrinol. & Metab.* **14**: 640, 1954.

8. YOUNT, E., and LITTLE, J. M.: Renal clearance in patients with myxedema, *J. Clin. Endocrinol. & Metab.* **15**: 343, 1955.
9. CORCORAN, A. C., and PAGE, I. H.: Specific renal functions in hyperthyroidism and myxedema, *J. Clin. Endocrinol.* **7**: 801. 1947.
10. SCHEINBERG, P.; STEAD, E. A., JR.; BRANNON, E. S., and WARREN, J. V.: Correlative observations on cerebral metabolism and cardiac output in myxedema, *J. Clin. Invest.* **29**: 1139, 1950.

THE EFFECTS OF CERTAIN PHYSICAL AND EMOTIONAL TENSIONS AND STRAINS ON FLUCTUATIONS IN THE LEVEL OF SERUM PROTEIN-BOUND IODINE

ROBERT VOLPÉ, M.D.*, JOAN VALE, M.D.† AND MACALLISTER W. JOHNSTON, M.D.

with the technical assistance of NORMA HUBER, R.N.

The Departments of Medicine, University of Toronto and Toronto General Hospital, Toronto, Ontario, Canada

ABSTRACT

The effect of physical and emotional tensions and strains on the fluctuation of the serum protein-bound iodine level was studied in groups of healthy people and in two groups of patients. The effect of the tension and strain of examination was observed in 11 medical students preparing for annual examinations and in 11 Royal College of Physicians candidates. The stress of athletic contest on the protein-bound iodine level was observed in 7 professional football players in training and during their scheduled games. There was no fluctuation beyond that found in normal healthy persons. Similarly, in 8 patients prior to and following major operations and in 9 patients following myocardial infarction, the fluctuations of the serum protein-bound iodine level remained within the usual normal range. Our data fail to provide any evidence that the function of the normal thyroid gland is readily affected by severe mental and physical stresses or strains.

IN THE last few years the subject of *stress* has received considerable attention. The rapid adrenal response to emotional and physical stimuli has been fairly well described and found to occur consistently, but conflicting reports appear in the literature about the response of the thyroid to similar influences. It has been suggested that hyperthyroidism may be precipitated by physical or emotional strains (1–6). This hypothesis remains unproved.

The effect of emotional stress has been studied by numerous investigators. Hetzel *et al.* (7) induced strong emotional reactions by interviews in 3 euthyroid and 3 hyperthyroid subjects. They found an initial slight fall in the serum protein-bound iodine (PBI) level during the interview period, immediately followed by a rise well above control values. These authors

Received July 30, 1959.

* Medical Research Fellow, National Research Council, Canada, 1955–57.

† Medical Research Fellow, National Research Council, Canada, 1953–55.

concluded that the thyroid gland is able to respond rapidly to acute emotional disturbance. Tingley *et al.* (8) studied the serum protein-bound iodine level in 11 medical students undergoing examinations. There were no significant variations of the values for protein-bound iodine on the control day but, on the day of the examination, they found a significant rise. In 28 patients, Dongier *et al.* (9) carried out 36 interviews designed to induce overt anxiety and found that the serum protein-bound iodine values were not affected. These investigators performed long-term studies on 25 volunteers and 27 patients with anxiety, 10 of whom had a family or personal history of thyrotoxicosis although none of the subjects had thyroid disease at the time of study. The subjects were given tracer doses of radioactive iodine (I^{131}) and the rate of disappearance of the substance from the thyroid gland was measured. There was no significant difference in thyroidal 24-hour I^{131} uptake and the biologic half-life of I^{131} between the control group and the anxious patients. However, in 12 anxious patients who were considered to have "personality structures similar to hyperthyroid patients," there was a shorter biologic I^{131} half-life when compared with the control group.

Board *et al.* (10) studied the concentration of serum protein-bound iodine in 20 moderately disturbed patients within twenty-four hours after their admission to a psychiatric ward. The mean value for serum protein-bound iodine concentration was distinctly higher than the average for normal people; there was a small decline in the values during the morning after admission. Fourteen patients were retested seventeen days later: the more disturbed patients, who had received electroshock therapy, had relatively high serum protein-bound iodine levels (still within the normal range), whereas the less disturbed patients, who had not received electroshock therapy, had normal levels.

Similar studies by Starr *et al.* (11) and Brody (12) showed no significant alterations in the serum protein-bound iodine level in psychiatric patients. Other observations of thyroid function in schizophrenia (13, 14) and periodic catatonia (15) have failed to define clearly the status of thyroid function in disturbed patients.

Since it has been established that administration of large doses of adrenocorticotropin (ACTH) or cortisone will reduce the basal metabolic rate, serum protein-bound iodine level and thyroidal I^{131} uptake (16–20), it seems surprising that stress would cause an increase in these indices; the reverse might be expected. In rabbits, Brown-Grant *et al.* (21) showed that "emotional stress" (produced by subcutaneous faradism, restraint or abrupt changes in environmental lighting) induces a prompt *inhibition* in the release of radioactive iodine-labeled hormone from the thyroid gland; similar inhibition of the thyroid was also produced by physical traumata.

In rats, acute stresses such as anoxia and physical trauma (*e.g.*, operations) result in a decreased uptake of radioactive iodine by the thyroid and a decreased serum protein-bound iodine level (22, 23). On the other hand, Ackerman and Arons (24) recently showed that the intravenous administration of epinephrine or norepinephrine to dogs results in a marked increase in the concentration of protein-bound I^{131} in thyroid vein blood. In one dog, chromatographic studies showed this increase in PBI^{131} to be due to rises in both thyroxine and triiodothyronine. In their paper, the authors cite the previous conflicting experimental reports which relate to the effects of administered adrenal medullary hormones on thyroid function in animals. Hanbury (25) has recently reviewed the literature regarding the effects of trauma (usually surgical) on thyroid function in man; although reports were conflicting, the consensus was that, following surgery, there was generally an increase in the level of serum thyroxine and an increase in the rate of peripheral degradation of thyroxine.

In view of the fact that physical and emotional trauma in animals and administration of corticosteroids in humans decrease thyroid function as measured by the foregoing indices, and because of the conflicting observations recorded in the literature, it was considered worth while to study the effects of emotional and physical stress on the serum protein-bound iodine level of a number of apparently healthy people and of patients undergoing serious surgical procedures or following acute myocardial infarction.

METHODS AND SUBJECTS

Methods

Estimation of serum protein-bound iodine concentration was carried out by a modification of the method of Barker *et al.* (26). In our hands (27), the normal range is between 3.5 and 6.5 μg. per 100 ml. (in 479 euthyroid persons) with a standard deviation of 0.717 μg. per 100 ml.

The range and standard deviation of multiple duplicate determinations on each of 9 samples of serum are recorded in Table 1. The standard deviation of *duplicate* determinations on 122 sera taken at random from our records is shown in Table 2; 75 of these sera came from euthyroid persons.

Since only a few determinations of thyroidal 24-hour I^{131} uptake were made in our study, these results were excluded from consideration. It was not practical in this particular series to study other parameters of thyroid function involving radioiodine studies, *e.g.*, PBI^{131} levels, thyroxine "turnover rates" and biologic half-life of radioactive iodine.

Subjects

Normal control group. Nine normal persons were studied for periods ranging between eighteen months and five years by repeated serum protein-bound iodine determinations. These subjects included doctors, nurses, secretaries and technicians; there were 4 males and 5 females ranging from 26 to 40 years of age. They were subject to the usual stresses and strains of their occupations, and represent a cross-section of emotional stability.

TABLE 1. VARIATION IN MULTIPLE DETERMINATIONS OF PROTEIN-BOUND IODINE CONCENTRATION IN SINGLE SAMPLES OF SERUM

Subject	No. of determinations on each sample	Serum PBI level (μg./100 ml.)			
		Mean	Range	Standard deviation	Standard error
N.H., #1	6	4.8	4.4–5.0	0.21	0.085
N.H., #2	5	5.0	4.7–5.2	0.24	0.017
N.H., #3	12	5.0	4.6–5.4	0.28	0.081
E.D.	6	3.2	2.6–3.5	0.33	0.135
M.J., #1	5	4.2	4.0–4.4	0.16	0.073
M.J., #2	5	4.1	3.8–4.8	0.49	0.22
J.V.	9	4.7	4.5–4.9	0.16	0.054
D.F.	7	4.9	4.6–5.4	0.34	0.128
J.L.	7	4.9	4.7–5.2	0.22	0.083

Persons under stress. In 11 undergraduate male medical students, the concentration of serum protein-bound iodine was determined repeatedly during an academic term and the period of annual examinations.

Eleven male candidates for the Royal College of Physicians examinations were followed for several weeks prior to the examinations, daily during the examination period, and for variable periods thereafter. In addition to the serum protein-bound iodine level, thyroidal I^{131} uptake was determined in 4 of these subjects.

Seven professional football players (Toronto Argonauts) were studied by means of serum protein-bound iodine determinations. With one exception, each was followed over

TABLE 2. VARIATION IN DUPLICATE DETERMINATIONS OF SERUM PROTEIN-BOUND IODINE IN LARGE NUMBERS OF SERA

Ranges studied (μg=/100 ml.)	Number of sera	Mean of difference	Range of difference	Standard deviation	Standard error	95% fiducial limit
1.2– 3.5	27	0.28	0.01–0.78	0.24	0.046	0.28±0.09
3.5– 6.5	75	0.41	0.02–1.44	0.33	0.038	0.41±0.08
7.5–16.0	20	0.62	0.05–3.53	0.84	0.188	0.52±0.37

Applying Student's "*t*" test, it may be shown that the percentage error of each of the three groups recorded is not significantly different from the others, *i.e.*, the accuracy of measurement of the serum protein-bound iodine is not affected by the concentration within the range 1.2 to 16.0 μg. per 100 ml.

a three-game period; one athlete was injured during the first game and thus was not followed through the subsequent two-game period. Blood samples were taken twice during the practice week as well as immediately following the game. The players would not submit to venipuncture immediately preceding the contest.

Eight hospitalized patients were studied prior to, during and after surgical procedures. In all instances the patient had been told of the gravity of the operation some days before the actual procedure. Although most patients had considerable discomfort for a few days postoperatively, all recovered reasonably well from the effects of the operation.

Nine patients admitted to the hospital immediately following acute myocardial infarction were studied. The first blood samples for determination of protein-bound iodine were taken at the time of admission; this varied from thirty minutes to six hours after the onset of chest pain. One patient died twelve hours after admission, and another died six days later after recurrent episodes of ventricular tachycardia.

RESULTS

Control group

Technical errors in the determination. The variation in multiple determinations of the serum protein-bound iodine level on each of 9 samples of serum is recorded in Table 1. The maximum number of determinations on an individual sample was 12. The accuracy of the method and technical error can be gauged from the recordings. Variations in determinations performed in duplicate only, on 122 samples of serum are shown in Table 2. These results were taken at random from our records and represent only a very small fraction of the total number of determinations carried out in this laboratory; it was considered that the random nature of the collection would yield a significant analysis. The results, as tabulated, again reveal the narrow range of technical error of the test in our laboratory.

Fluctuation of the serum protein-bound iodine level in normal subjects over a long period of time. This is shown in Table 3. Frequent measurements of

TABLE 3. FLUCTUATION OF THE SERUM PROTEIN-BOUND IODINE LEVEL IN NORMAL SUBJECTS OVER LONG PERIODS OF TIME

Subject		No. of tests	Total period	Serum PBI level (μg./100 ml.)		
Name	Age & Sex			Mean values	Variation	Standard deviation
N.H.	36 F	132	4 yrs.	4.6	3.5–5.8	0.72
J.V.	28 F	83	3 yrs.	4.8	4.2–6.1	0.34
C.E.	31 M	26	4 yrs.	5.5	4.7–6.5	0.52
R.V.	30 M	20	18 mos.	5.5	4.7–6.2	0.42
E.D.	29 F	15	3 yrs.	4.9	4.5–5.8	0.48
M.S.	29 F	15	3 yrs.	5.0	4.2–5.8	0.48
K.C.	43 F	11	2 yrs.	4.8	4.5–5.1	0.23
R.S.	30 M	10	3 yrs.	4.5	3.9–6.6	0.77
M.J.	36 M	11	4 yrs.	4.3	4.1–4.9	0.24

serum protein-bound iodine concentration were made over long periods of time on 9 normal persons (5 doctors, 1 nurse, 2 secretaries and 1 laboratory technician) who were carrying on ordinary activity. The number of tests on each varied between 10 and 132 over periods ranging from eighteen months to four years. The range of fluctuation varied between 0.8 and 2.7 μg. per 100 ml. The fluctuations could not be correlated with the intercurrent (mild) stresses and strains which most of the subjects experienced during the study period. The fluctuations in every subject remained well within the normal range. On statistical examination, however, they often exceeded the technical error of the test in the range of 3.5 to 6.5 μg. per 100 ml. (Table 2). Thus, these variations were significant, though small.

Persons under stress

Effect of the stress of annual examinations on the serum protein-bound iodine level of 11 male medical undergraduate students. Repeated serum protein-bound iodine determinations were made on 11 male undergraduate students, beginning in the autumn term and extending through the period of spring (final) examinations. The results are listed in Table 4. These young men were of average emotional stability and, except for a period

TABLE 4. EFFECT OF THE STRESS OF ANNUAL EXAMINATIONS ON THE SERUM PROTEIN-BOUND IODINE LEVEL OF 11 MALE MEDICAL UNDERGRADUATE STUDENTS

Time	Serum PBI level (μg./100 ml.)		
	Range	Mean	Standard deviation
Before examinations			
7 months	3.9–5.7	4.95	0.53
6 months	3.7–5.4	4.95	0.66
5 months	3.8–5.8	4.72	0.54
4 months	3.5–5.2	4.26	0.61
3 months	3.8–5.2	4.4	0.57
2 months	3.9–5.3	4.6	0.42
1 month	3.2–5.3	4.8	0.71
2 weeks	3.6–5.1	4.5	0.57
Immediately after			
First examination	3.1–5.7	4.9	0.93
Fourth examination	3.4–6.4	5.4	0.81
Last examination	3.6–6.1	4.9	1.03
After all examinations			
1 day	3.6–5.9	5.2	0.82
3 days	3.8–5.8	4.7	0.9

preceding and during the final examinations, were under no unusual tensions. For several days prior to the examinations, however, each subject felt considerable anxiety and most of them had mild to moderate symptoms of autonomic disturbance, *e.g.*, sweating, tachycardia, nervousness, epigastric discomfort and diarrhea. These were accentuated on the morning of the first examination. Following the last examination there was a short period of one to two days during which the subject felt "let-down" and experienced vague depression, apathy and restlessness.

During this entire period, the fluctuations in the concentration of protein-bound iodine in these subjects were within the range of those for the control group. In 9 of the 11 subjects, however, the serum protein-bound iodine level was at its highest level during the examination period; the elevation was very slight in most instances, and was of no significance in any. It is thus apparent that the tension of examinations had no significant effect on the levels of the serum protein-bound iodine in these students.

The effect of temporary anxiety on the serum protein-bound iodine level in 11 male candidates for the Royal College examinations is recorded in Table 5. This group of subjects was composed of 11 physicians, all of whom had completed at least five years of postgraduate work. The Royal College examinations represented to them a very difficult obstacle which they must overcome in order to practice their specialty. Because of the high failure rate, each candidate was very anxious about the outcome since he considered his future to be hanging in the balance. Because of this severe anxiety there were moderate psychosomatic disturbances in all subjects for several days prior to the examinations. These varied from mild sweating, tachycardia, restlessness, insomnia and apprehension to severe epigastric distress, marked diarrhea and frequency of micturition. These symptoms abated moderately after the first examination and cleared quickly following the last written examination.

Samples of blood for protein-bound iodine estimations were drawn from each candidate every week for several weeks prior to the examinations, on the morning of each examination and for a short period following the last examination. With 2 possible exceptions, no trends could be detected; the protein-bound iodine values were not appreciably altered during the period of stress when compared with those of the early pre-examination period. In 2 subjects the protein-bound iodine values did reach their highest levels during the period of anxiety; however, these elevations were very slight and did not exceed the normal fluctuation when compared with others in the same group or in the control group (Table 3).

The effect of the physical and emotional stress of professional football contests on the level of serum protein-bound iodine was studied in 6 players through a series of three games, and a seventh player was studied for one

TABLE 5. EFFECT OF TEMPORARY ANXIETY ON THE SERUM PROTEIN-BOUND IODINE LEVEL IN 11 MALE CANDIDATES FOR THE ROYAL COLLEGE EXAMINATIONS

Time	Serum PBI level (μg./100 ml.)		
	Range	Mean	Standard deviation
Before examinations			
10 weeks	3.8–6.0	4.6	0.87
9 weeks	4.5–5.7	5.0	0.51
8 weeks	4.0–5.5	4.6	0.73
7 weeks	—	—	—
6 weeks	4.5–6.2	5.4	0.70
5 weeks	—	—	—
4 weeks	3.9–6.1	4.8	0.66
3 weeks	3.8–6.3	5.1	0.72
2 weeks	4.0–5.5	4.9	0.60
1 week	4.3–6.2	4.5	0.64
2 days	4.1–5.2	4.7	0.48
1 day	4.1–5.6	4.7	0.62
Day of:			
First examination	4.2–5.7	5.0	0.53
Second examination	4.1–6.0	4.9	0.60
Third examination	4.3–5.8	4.9	0.60
After examinations			
2 days	4.3–5.2	4.9	0.33
1 week	4.3–6.0	5.0	0.95
2 weeks	3.8–5.9	5.1	0.70
3 weeks	4.3–5.6	4.8	0.50
4 weeks	4.7–6.0	5.1	0.71

game only. (An injury sidelined this athlete from further participation in the games.) Interested in their professional status, these young men took their athletic duties very seriously. Before each contest the players were tense and restless; the anxiety cleared almost immediately after the opening whistle. The particular players chosen were "first-string-regulars" who played a considerable part of each match; on each occasion, these men were quite exhausted at the end of the game. The three-game period was actually a crucial part of the season; the team won only one game of the three, losing the other two. After the two losses the dressing-room was a scene of utter dejection shared by every member of the team; on the other hand, the one win was followed by marked elation.

Samples of blood were taken twice during the week and then as soon as the game was over. Blood could not be taken prior to the game because, in

their very tense state, they would not submit to venipuncture at that time.

During the entire period of observation, the serum protein-bound iodine values remained within the normal variation, as shown in Table 6. The level of the serum protein-bound iodine was not affected significantly in blood taken immediately after the games.

Effect of physical and emotional stress of operative procedures on the level of serum protein-bound iodine. Eight patients who underwent major operations were studied. They varied in age from 29 to 67 years and included 5 males and 3 females. Samples of blood were taken a few days before the operation, immediately before and immediately after the procedure, and

TABLE 6. EFFECT OF PROFESSIONAL FOOTBALL GAMES ON THE SERUM PROTEIN-BOUND IODINE LEVEL OF 7 PLAYERS*

Date (1956)	Comment	Serum PBI level (μg./100 ml.		
		Range	Mean	Standard deviation
Oct. 17	Before practice	3.5–5.9	4.7	0.83
Oct. 19	After practice	3.8–5.8	4.9	0.68
Oct. 20	After a game	4.4–5.6	4.9	0.45
Oct. 22	Before practice	3.5–5.0	4.5	0.63
Oct. 25	After practice	4.0–5.6	4.9	0.61
Oct. 27	After a game	4.2–5.3	4.6	0.41
Oct. 29	Before practice	3.5–5.1	4.4	0.59
Nov. 1	After practice	3.6–5.6	4.8	0.71
Nov. 3	After a game	4.0–5.4	4.6	0.58

* One player dropped out of study after one game (see text).

for some days following the operation. Thyroidal radioactive iodine uptakes were determined preoperatively and postoperatively. The results of this study are recorded in Table 7.

All patients had been told about their operation in advance and they evinced varying degrees of apprehension. However, each patient accepted the necessity of the operation and was optimistic about the outcome. Psychosomatic signs and symptoms were not prominent prior to the operation. With 2 exceptions the postoperative course was relatively uneventful. One patient went into a state of shock towards the end of the operative procedure but was soon resuscitated by blood transfusion; he suffered atelectasis of the left lower lobe in the immediate postoperative period. In another patient, the "post-commissurotomy syndrome" with fever, chest pain and pericarditis developed four days after mitral commissurotomy. In these 2 patients, as in all but 1 of the entire group, there were no unusual

TABLE 7. EFFECT OF THE STRESS OF MAJOR OPERATIONS ON THE SERUM PROTEIN-BOUND IODINE LEVEL IN 8 PATIENTS

Time	Serum PBI level (μg./100 ml.)		
	Range	Mean	Standard deviation
Before operation			
3 days	3.9–6.0	5.11	0.80
2 days	3.8–5.2	4.72	0.53
1 day	4.0–5.5	4.94	0.59
immediately	4.2–5.7	5.18	0.46
After operation			
immediately	4.0–6.0	5.21	0.66
1 day	3.6–5.6	4.93	0.78
2 days	4.2–5.8	5.23	0.71
3 days	4.0–6.2	5.10	0.89
4 days	4.2–5.2	4.83	0.55
5 days	5.1–5.8	5.50	0.49
6 days	3.8–5.7	5.00	0.74
7 days	4.2–5.4	5.00	0.56

alterations either preoperatively or postoperatively in the levels of serum protein-bound iodine. Only in 1 patient, who had a gastrectomy with an uneventful postoperative course, did the level rise slightly. In any event the "rise" did not exceed the normal fluctuation.

Only 2 thyroidal radioactive iodine uptakes were determined on each patient—one preoperatively, and the other approximately two weeks after operation. The variations were within the normal fluctuations.

The effect of the physical and emotional stress of myocardial infarction on the level of serum protein-bound iodine in 9 patients is recorded in Table 8. The 9 patients were all admitted to the hospital immediately following acute myocardial infarction; their ages varied from 42 to 69 years. The first specimens of blood for protein-bound iodine estimation were obtained thirty minutes to six hours following the onset of pain. One patient died within twelve hours; another had recurrent ventricular tachycardia and died on the sixth hospital day. The remaining 7 patients recovered uneventfully, although 1 has had increased angina pectoris after a second myocardial infarct.

During the periods of observation there were no alterations beyond the normal fluctuation levels of the serum protein-bound iodine and no trends in either direction.

TABLE 8. EFFECT OF STRESS OF MYOCARDIAL INFARCTION ON THE SERUM PROTEIN-BOUND IODINE LEVEL IN 9 PATIENTS*

Time	Serum PBI level (μg./100 ml.)		
	Range	Mean	Standard deviation
On admission	3.9–5.8	4.88	0.62
2nd day	4.0–6.2	4.98	0.79
3rd day	4.2–6.1	4.91	0.79
5th day	4.7–6.1	5.26	0.48
7th day	3.8–5.9	4.91	0.67
10th day	4.1–6.0	4.73	0.68
14th day	3.9–6.0	5.04	0.68
21st day	4.1–5.7	4.91	0.51
28th day	4.1–5.6	4.91	0.50
35th day	4.0 5.9	4.8	0.59

* Two patients died during study (see text) but their available values are included.

DISCUSSION

It is evident that there is a small fluctuation in the serum concentration of protein-bound iodine in normal persons from time to time, which is often greater than the technical error of the test. The degree of variation and its level within the normal range differs from person to person. In each subject in the series, the fluctuation was apparently independent of the normal day-to-day stresses which affect everyone. Major emotional influences (for example, family illness) did not appear to affect the fluctuations. Margolese and Golub (28) found a cyclic variation in menstruating women but, as in our studies, the total fluctuation was not greater than in men. The diurnal pattern of serum protein-bound iodine has been studied in our laboratory in 7 normal subjects. As will be reported elsewhere (29), no significant diurnal fluctuation was noted; specimens were taken at 8:30 A.M. 1:30 P.M., 5:30 P.M. and 3:30 A.M. This is in accord with the findings of Tingley and his associates (8) who, however, did not take night samples although they took them more frequently during the day.

In the group of undergraduate students, the highest levels of protein-bound iodine recorded were during the period of examinations. All were within normal limits however, and the mean variation was not greater than 1.0 μg. per 100 ml. of serum over the whole period. Among the graduate students (Royal College candidates), only 2 showed a suggestion of a rise during the period of examination. Again, this change was well within the range of normal fluctuation and was not significant. In the remainder of these candidates there were no discernible trends. Among the 7 professional

athletes, the serum protein-bound iodine levels showed fluctuations which could not be correlated with the actual stress of the games; these fluctuations again were normal in their amplitude. In the group of patients undergoing major surgery, all but one showed minor variations which could not be correlated with the stress of the operation; 2 of these patients had complicated postoperative courses and yet there was no significant change in the concentration of serum protein-bound iodine. In 1 patient there appeared to be a slight increase in protein-bound iodine values concomitant with the surgical procedure; this change was not great, being actually within the range of normal fluctuation; thus it was not significant. Similarly, no significant changes in values were observed in the patients who had suffered acute myocardial infarction.

It is possible that more frequent determinations of the serum protein-bound iodine concentration in these various groups of subjects might have demonstrated greater temporary variations in the levels, but this does not seem probable. It is also possible that a larger group of cases might have included persons whose thyroid function might have been affected by such stresses and strains. Studies of the *dynamics* of thyroid function (*e.g.*, thyroxine "turnover rates") might well yield different results in such groups of subjects.

In the early phase of this study it was thought that variations in the concentration of serum protein-bound iodine might be affected by ordinary emotional stresses of life, such as family illnesses, but this proved to be wrong. No correlation was found between the PBI fluctuations and the ordinary stresses of living. No environmental factors were found to influence or explain the normal variation. Similarly, in all the groups studied the variations in the serum protein-bound iodine level fell within the normal fluctuation. In the group of undergraduate students there appeared to be a very slight trend towards higher normal values during the examination period; and, in addition, 2 Royal College candidates showed their highest values during the examination period. In none of the others, however, was there a trend in either direction. It was not possible to correlate the fluctuations found with the particular stress or strain encountered. Even the complicated postoperative courses of 2 patients failed to affect the protein-bound iodine levels significantly. The same was true for the physical and mental trauma of myocardial infarction.

It may be hypothesized that, during the early stages of hyperthyroidism before it is possible to measure any definite physiologic change in this condition, the serum protein-bound iodine level might be affected by such stressful experiences. Within the limits of this study, however, it is obvious that the concentration of serum protein-bound iodine in the various groups

of patients and healthy persons was not significantly affected by the stress and strains of examinations, athletic contests, major surgical procedures or myocardial infarcts.

Acknowledgments

Grateful acknowledgment is made to Professor R. F. Farquharson for his advice, criticism and stimulus, and to Dr. L. Lax for his statistical review.

REFERENCES

1. Mittelmann, B.: Psychogenic factors and psychotherapy in hyperthyreosis and rapid heart imbalance, *J. Nerv. & Ment. Dis.* **77:** 465 (May) 1933.
2. Conrad, A.: The psychiatric study of hyperthyroid patients, *J. Nerv. & Ment. Dis.* **79:** 505 (June) 1934.
3. Lidz, T.: Emotional factors in the etiology of hyperthyroidism, *Psychosom. Med.* **11:** 2 (Jan.–Feb.) 1949.
4. Ham, G. C.; Alexander, F., and Carmichael, H. T.: Dynamic aspects of the personality features and reactions characteristic of patients with Graves' disease, *in* Life, Stress and Bodily Disease, *Research Publ. Assn. Res. Nerv. & Ment. Dis.* **29:** 451 (Dec.) 1949.
5. Dobyns, B. M.: Physiologic concepts in the diagnosis and treatment of Graves' disease, *Am. J. Med.* **20:** 684 (May) 1956.
6. Ferguson-Rayport, S. M.: The relation of emotional factors to recurrence of thyrotoxicosis, *Canad. M. A. J.* **75:** 993 (Dec. 15) 1956.
7. Hetzel, B. S.; de la Haba, D. S., and Hinkle, L. E., Jr.: Rapid changes in plasma PBI in euthyroid and hyperthyroid subjects, *Tr. Am. Goiter A.* 1952, p. 242.
8. Tingley, T. O.; Morriss, A. W., and Hill, S. R.: Studies on diurnal variation and responses to emotional stress to the thyroid gland, *Clin. Res. Proc.* **1:** 134, 1958.
9. Dongier, M.; Wittkower, E. D.; Stephens-Newsham, L., and Hoffman, M. M.: Psychophysiological studies in thyroid function, *Psychosom. Med.* **18:** 310 (July-Aug.) 1956.
10. Board, F.; Persky, H., and Hamburg, D. A.: Psychological stress and endocrine functions, *Psychosom. Med.* **18:** 324 (July–Aug.) 1956.
11. Starr, P.; Petit, D. W.; Chaney, A. L.; Rollman, H.; Aiken, J. B.; Jamieson, B., and Kling, I.: Clinical experience with the blood protein-bound iodine determination as a routine procedure, *J. Clin. Endocrinol.* **10:** 1237 (Oct.) 1950.
12. Brody, E. B.: Psychological tension and serum iodine levels in psychiatric patients without evidence of thyroid disease, *Psychosom. Med.* **11:** 70 (Mar.–Apr.) 1949.
13. Bowman, K. M.; Miller, E. R.; Dailey, M. E.; Simon, A., and Mayer, B. F.: Thyroid function in mental disease; a multiple test survey, *J. Nerv. & Ment. Dis.* **112:** 404 (Nov.) 1950.
14. Cranswick, E. H.: Tracer iodine studies on thyroid activity and thyroid responsiveness in schizophrenia, *Am. J. Psychiat.* **112:** 170 (Sept.) 1955.
15. Gornall, A. G.; Eglitis, B.; Miller, A.; Stokes, A., and Dewan, J. G.: Long-term clinical and metabolic observations on periodic catatonia, *Am. J. Psychiat.* **109:** 584 (Feb.) 1953.
16. Hill, S. R., Jr.; Reiss, R. S.; Forsham, P. H., and Thorn, G. W.: The effect of adrenocorticotropin and cortisone on thyroid function: thyroid-adrenocortical interrelationships, *J. Clin. Endocrinol.* **10:** 1375 (Nov.) 1950.

17. WOLFSON, W. Q.; BEIERWALTES, W. H.; ROBINSON, W. D.; DUFF, I. F.; JONES, J. R.; KNORPP, C. T., and EYA, M.: Corticogenic hypothyroidism: its regular occurrence and clinical significance during prolonged therapeutic administration of ACTH or cortisone, *J. Lab. & Clin. Med.* **36:** 1005 (Dec.) 1950.
18. FREDRICKSON, D. S.; FORSHAM, P. H., and THORN, G. W.: The effect of massive cortisone therapy on measurements of thyroid function, *J. Clin. Endocrinol.* **12:** 541 (May) 1952.
19. HARDY, J. D.; RIEGEL, C., and ERISMAN, E. P.: Experience with the protein-bound iodine (PBI); the effect of ACTH and cortisone on thyroid function, *Am. J. M. Sc.* **220:** 290 (Sept.) 1950.
20. INGBAR, S. H., and FREINKEL, N.: ACTH, cortisone and the metabolism of iodine, *Metabolism* **5:** 652 (Nov.; Part 1) 1956.
21. BROWN-GRANT, K.; HARRIS, G. W., and REICHLIN, S.: The effect of emotional and physical stress on thyroid activity in the rabbit, *J. Physiol.* **126:** 29 (Oct.) 1954.
22. BOGOROCH, R., and TIMIRAS, P.: The response of the thyroid gland of the rat to severe stress, *Endocrinology* **49:** 548 (Oct.) 1951.
23. VAN MIDDLESWORTH, L., and BERRY, M. M.: Iodide metabolism during anoxia, nephrectomy, trauma, avitaminoses and starvation in the rat, *Am. J. Physiol.* **167:** 576 (Dec.) 1951.
24. ACKERMAN, N. B., and ARONS, W. L.: The effect of epinephrine and nor-epinephrine on the acute thyroid release of thyroid hormones, *Endocrinology* **62:** 723 (June) 1958.
25. HANBURY, E. M.: Thyroid function after trauma in man, *Metabolism* **8:** 904 (Nov.) 1959.
26. BARKER, S. B.; HUMPHREY, M. J., and SOLEY, M. H.: The clinical determination of protein-bound iodine, *J. Clin. Invest.* **30:** 55 (Jan.) 1951.
27. HUBER, N.: The difficulties encountered in the measurement of the serum protein-bound iodine and the value of this test in the assessment of thyroid function, *Canad. J. Med. Tech.* **18:** 77 (Sept.) 1956.
28. MARGOLESE, M. S., and GOLUB, O. J.: Daily fluctuations in serum protein-bound iodine level, *J. Clin. Endocrinol. & Metab.* **17:** 849 (July) 1957.
29. SCHATZ, D. L., and VOLPÉ, R.: Lack of diurnal variation in the level of serum protein-bound iodine, *J. Clin. Endocrinol. & Metab.* **19:** 1495 (Nov.) 1959.

PARADOXICAL LENGTHENING OF MUSCLE CONTRACTION TIME IN HYPOTHYROID PATIENTS GIVEN SMALL DOSES OF *l*-TRIIODOTHYRONINE, THYROXINE AND DESICCATED THYROID*

ARTHUR S. WEISSBEIN, CAPTAIN, MC, USA† AND
JOHN D. LAWSON, MAJOR, MC, USA‡
Brooke Army Hospital, Fort Sam Houston, Texas

ABSTRACT

Since the prolongation of the Achilles reflex associated with myxedema is shortened during treatment with thyroid hormone, it was surprising to find that when *small* doses of thyroid hormone were administered to hypothyroid patients, the reflex time became longer rather than shorter. Further investigation revealed that this paradoxical response occurred in all patients who were hypothyroid by standard tests of thyroid function. Several borderline hypothyroid patients were also studied. The results thus far suggest that this paradoxical muscular response may possibly provide a means for detection of milder degrees of hypothyroidism than are demonstrable by the standard clinical tests now employed. The test was standardized on 40 hypothyroid patients. It was found that the most economical and simple method consists of recording the duration of the Achilles reflex contraction time within twenty-four hours after administration of 25 μg. of *l*-triiodothyronine (liothyronine) in fractional doses.

SINCE Chaney's original quantitation of the prolonged Achilles reflex in myxedema (1), a number of investigators have corroborated his findings (2–4) and it has been noted that the Achilles reflex time becomes shorter as the patient approaches euthyroidism during treatment (1–4).

During the past four years we have consistently observed an unusual reflex phenomenon occurring in hypothyroid patients when therapy was instituted with *small* doses of thyroid, thyroxine or *l*-triiodothyronine (liothyronine). Initially, the reflex contraction time was lengthened, rather than shortened, as we expected. These observations and further studies form the basis of this report.

Received August 4, 1959.

* Presented in part at the meeting of the American College of Physicians in Chicago, Ill., April, 1959.

† *Present address:* 28th General Hospital, APO 219, New York, N. Y.

‡ *Present address:* Medical Service, U. S. Army Hospital, Fort Rucker, Ala.

MATERIALS AND METHODS

The Kinemometer (4) was used to record the Achilles reflex. The modification introduced in this study was recently described by us in another publication (5). The modification, now a standard part of the Kinemometer, consists of an electrical filter. The addition of the filter was necessitated by the fact that, although motion pictures of the Achilles reflex had shown the muscle movement to be smooth, secondary vibrations of the associated tendons were superimposed on the curve. This factor unduly complicated precise reading of the reflexes, particularly in severe hypothyroidism. With the use of the filter these vibrational artifacts were virtually eliminated. The simplification in reading the reflex introduced by the addition of the filter can be readily appreciated in the tracings shown in Figures 1 and 2. With unfiltered reflexes, in recording greatly prolonged responses, there is a tendency to interpret the contraction phase as being much shorter than it actually is, due to vibratory artifacts. For example, in Figure 2, the true end of contraction is marked in the top unfiltered tracings, and it is clear that considerable error could be made in estimating the contraction interval. Practically, in diagnosis, this is inconsequential, but in following the effects of therapy quantitatively it could be critical. The filter, now employed routinely, eliminates these difficulties. The displacement device recently reported by Gilson (6) for recording the Achilles reflex appears to obviate the same difficulties, although our experience with his device is limited.

The ranges of contraction times, *using the filter*, were as follows: in euthyroid subjects, 190–280 milliseconds; in hyperthyroid patients, 144–208 milliseconds; and in hypothyroid patients, 270–480 milliseconds. The *actual* contraction times were consistently 40 milliseconds shorter than those recorded with the filter. This 40-millisecond increment (one small square at standard electrocardiographic speed) relates to the condenser charge time of the filter and is constant irrespective of the voltages produced or the rapidity of change of the generated voltages (5). If the 40-millisecond prolongation due to use of the filter is subtracted from the foregoing ranges, the resulting values are not significantly different from the normal values previously reported (4), with the single exception that the hypothyroid range has widened toward longer reflexes.

All the reflexes were recorded with a standard electrocardiograph with a paper speed of 25 mm. per second. A minimum recording of 20 reflexes was made for each patient and the shortest reflex was read in establishing the value for a particular patient. To determine the variability of the reflexes, 100 patients in various diagnostic categories underwent 6 separate reflex determinations approximately one hour apart on the same day. The maximum reflex variation when the "shortest reflex" criterion was used was ± 8 milliseconds. This test of variation is considered to be more valid than the "average reflex" criterion previously reported (7), since the objective is optimum performance. Practically, either criterion can be used, but the "shortest reflex" criterion is simple, accurate, reproducible, and appears to be the most reasonable. All the reflexes in this study were "filtered," although unfiltered reflexes were also recorded for possible future reference.

RESULTS

Following the initial observation of paradoxical lengthening of the reflex time in 10 myxedematous patients treated with $\frac{1}{8}$ grain of desiccated thyroid daily, we decided to determine the response of myxedematous patients to the daily administration of 12.5 μg. of liothyronine (6 cases) or

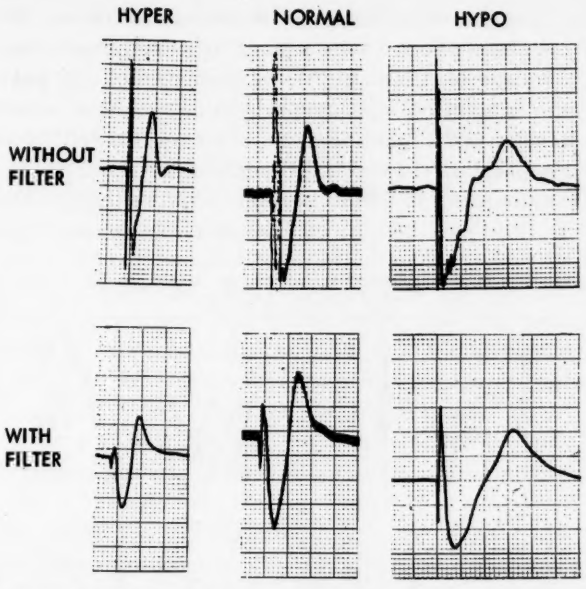


FIG. 1. Recordings of Achilles reflex in hyperthyroidism, euthyroidism and hypothyroidism. Note 40 millisecond lag between unfiltered and filtered recordings.

25 μg. of thyroxine (7 cases). In each instance the reflex contraction time was lengthened between 30 and 168 milliseconds. Five of the patients were studied while taking all 3 of the thyroid compounds, at different times. Although there was no significant quantitative difference in the response to the three preparations, it was noted that the prolongation of the reflex occurred within twenty-four hours when liothyronine was used, within seven days when thyroxine was used, and within ten days when desiccated thyroid was used.

There was considerable variation in the time of the appearance of the initial prolongation with thyroxine (three to seven days) and desiccated thyroid (five to ten days), but with liothyronine the response uniformly appeared within twenty-four hours (usually within four hours).

Six patients with hyperthyroidism were then tested after the administration of 12.5 μg. of liothyronine daily for three days. No prolongation or shortening of their reflex time was noted at any time during the three days.

Forty hypothyroid (nonmyxedematous) patients were then given 12.5 μg. of liothyronine. These patients were clearly hypothyroid as judged by at least two standard tests of thyroid function, which included the serum protein-bound iodine (PBI) level, thyroidal I^{131} uptake, I^{131} conversion ratio, and basal metabolic rate (BMR). In each case the reflex con-

traction time lengthened at least 30 milliseconds (range, 30–120 milliseconds) within twenty-four hours, without a second reinforcing dose.

Following the lead of Adelman (8), we then studied 109 patients whose symptoms were compatible with hypothyroidism but in whom results of standard tests of thyroid function were either inconsistent or low-normal. Thirty of these patients showed a prolongation of the Achilles reflex contraction time amounting to 30 milliseconds or more (range, 30–60) following administration of 12.5 μg. of liothyronine. Seventy-nine patients

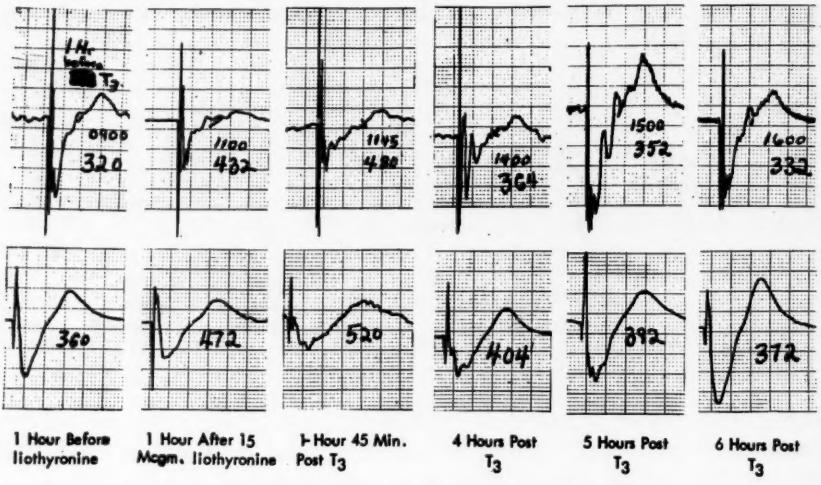


FIG. 2. Typical paradoxical lengthening of reflex response following administration of 12.5 μg. of liothyronine in a myxedematous patient.

showed no response. *All* were given a therapeutic trial of liothyronine for two months.

Of the 30 borderline hypothyroid patients showing lengthening of the reflex time, 27 were objectively improved. Of the 79 who showed no prolongation of the reflex time initially, 3 were objectively improved. We do not feel justified in drawing any firm conclusions from these results, since without other confirmatory evidence of abnormal thyroid function we cannot demonstrate conclusively that the patients who responded to treatment were indeed abnormal. The regularization of menses, disappearance of clots during menstrual flow, changes in cornification of the vaginal epithelium, decrease in the amount of falling hair, and changes in eyeglass prescriptions in varying degrees in the 30 patients recorded as objectively improved, and the distribution of improvement between the two groups, constitute an encouraging stimulus to further investigation.

In Figure 1 are shown typical reflexes (both filtered and unfiltered) in

hyperthyroidism, euthyroidism and hypothyroidism.

In Figure 2 the typical results of a liothyronine tolerance test are presented.

In Figure 3 are shown the typical initial paradoxical responses in 3 cases of hypothyroidism, along with subsequent reflex contraction times as therapy progressed. A 40-millisecond "blank" was subtracted from the filtered reflex values in order to obtain the *actual* reflex contraction time.

E.O. (Fig. 3) was a 45-year-old female who had signs and symptoms consistent with myxedema; her serum cholesterol level was 326 mg. per 100 ml., BMR −20 per cent, and serum PBI level 2.5 μg. per 100 ml. The initial contraction time was 260 milliseconds, and this rose to 290 milliseconds following administration of 12.5 μg. of liothyronine daily. She eventually became euthyroid clinically while taking 2 grains of thyroid daily; the contraction time was then 200 milliseconds.

D.Y. (Fig. 3) was a 56-year-old male who had frank myxedema with electrocardiographic changes and a PBI level of 2.3 μg. per 100 ml. His 48-hour thyroidal I^{131} uptake was 3.0 per cent, with a conversion ratio of 4.0 per cent. His BMR was +9 per cent. Again, the contraction time lengthened following daily dosage with 12.5 μg. of liothyronine, and subsequently became normal with 3 grains of thyroid daily.

I.B. (Fig. 3) was a 65-year-old female with classic myxedema. Her serum PBI level was 1.3 μg. per 100 ml., BMR −11 per cent, and serum

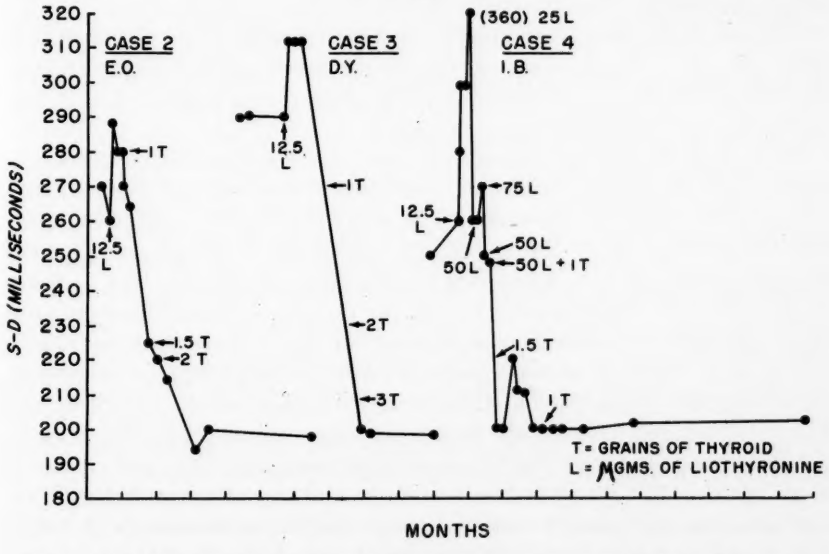


FIG. 3. Typical responses of reflexes during treatment, showing initial lengthening and final return to normal in 3 hypothyroid patients.

cholesterol level 380 mg. per 100 ml. Contraction time lengthened from 260 to 320 milliseconds following liothyronine therapy and eventually she became asymptomatic while taking only 1 grain of thyroid daily.

DISCUSSION

As this study progressed it became clear that the paradoxical lengthening of the Achilles reflex contraction time was a constant finding in all our patients demonstrated to be hypothyroid by conventional tests of thyroid function. This response may possibly provide a means of detecting subnormal metabolic states which cannot be confirmed consistently with the standard clinical tests now used. Whether or not the response in borderline patients can be interpreted as detecting "subclinical" hypothyroidism, will have to await the test of time.

At present, the standardized test is as follows: After a baseline tracing is taken, the patient is given 12.5 μg. of liothyronine by mouth at bedtime and again at seven o'clock the following morning. The reflex is recorded between 10 and 11 o'clock the same morning. The reason for the second dose is that several patients with unequivocally prolonged responses during the first ten hours of observation had shown only an equivocal prolongation twelve hours after a single dose of liothyronine; hence these positive results could be missed unless hourly tracings were taken. The difficulty of obtaining a number of reflex responses can be obviated by reinforcement with an early morning dose of 12.5 μg. of liothyronine three or four hours before the second reflex tracing is made.

The reason for finally standardizing the test with liothyronine rather than with other preparations was due to the more rapid clinical response, the saving of time, and the assurance of uniform dosage, rather than to any feeling that the drug had unique properties.

We have no explanation for the different times of onset of the reflex prolongation that occurred with the 3 compounds studied, nor have we any explanation for the paradoxical prolongation itself. It is tempting to postulate that it may be mediated through changes in TSH release from the pituitary, and that with decreasing thyroid function the pituitary becomes more sensitive to suppression with a given dose of thyroid. This would not be inconsistent with the data of others. For example, Werner has shown that it takes hundreds of micrograms of liothyronine to suppress minimally the I^{131} uptake of the thyroid gland in hyperthyroidism (9), and Starr and Liebhold-Schueck have shown that in normal subjects given only 8 μg. of liothyronine daily for six days there is a demonstrable, but small, decrease in I^{131} uptake (10). We have been unable to find any reference in the literature unequivocally demonstrating a greater *percentage* decrease in thyroidal I^{131} uptake following the administration of exogenous thyroid hormone in hypothyroidism compared to euthyroidism, as might be postulated if our hypothesis holds within the myxedema range. We plan to study the possi-

bility of an increased pituitary sensitivity to suppression by small doses of liothyronine in borderline or clearly hypothyroid patients.

It may be that along with the lengthening of the Achilles reflex a temporary, relative degree of hypometabolism may be induced iatrogenically with exogenous doses of liothyronine which are inadequate for treatment but quite effective in suppressing endogenous synthesis or release of pituitary hormone.

Addendum

The reader may be confused by the different dosage schedules of liothyronine mentioned in the text. To clarify any apparent discrepancies, it should be noted that *all* patients showing a paradoxical lengthening of the reflex following the administration of liothyronine demonstrated this prolongation within twenty-four hours after *only 12.5 Gm.* had been given. However, the time of occurrence of the prolongation and its duration was variable throughout the 24-hour period; thus frequent testing was required throughout the first twenty-four hours. Although such frequent testing can be carried out under investigative conditions, the time required under routine conditions would tend to be prohibitive.

When it was found that the onset of reflex lengthening could be *predictably anticipated* three to four hours after a second dose of liothyronine given eight to ten hours after the first dose, the standardized test was developed concurrently. This required only two reflex tests, and there was no sacrifice of accuracy. Hence, the administration of two 12.5-Gm. doses of liothyronine, though not required, proved to be the most convenient for both patient and physician. This dosage is now routinely used in our clinics.

REFERENCES

1. Chaney, W. C.: Tendon reflexes in myxedema: a valuable aid in diagnosis, *J.A.M.A.* **82**: 2013–2016, 1924.
2. Lambert, E. H.; Maderos, L. O., and Kelsey, M. P.: A study of the ankle jerk in myxedema, *Fed. Proc.* **7**: 68, 1948.
3. Harrell, G. T., and Daniel, D.: Delayed relaxation of tendon reflexes as an aid in the diagnosis of myxedema, *N. Carolina Med. J.* **2**: 549–551, 1941.
4. Lawson, J. D.: The free Achilles reflex in hypothyroidism and hyperthyroidism, *New England J. Med.* **259**: 761–764, 1958.
5. Lawson, J. D., and Weissbein, A. S.: The free Achilles reflex during treatment of hyperthyroidism, *Am. J. M. Sc.* **238**: 39–44, 1959.
6. Gilson, W. E.: Achilles-reflex recording with a simple photomograph, *New England J. Med.* **260**: 1027–1028, 1959.
7. Lawson, J. D.: The free Achilles reflex as an aid in the diagnosis of thyroid dysfunction, *U. S. Armed Forces Med. J.* **10**: 16–21, 1959.
8. Adelman, J. A.: Scientific Exhibit, A.M.A. Meeting, San Francisco, California, June, 1959.
9. Werner, S. C.: Responses to tri-iodothyronine as index of persistence of disease in thyroid remnant of patients in remission from hyperthyroidism, *J. Clin. Invest.* **35**: 57–61, 1956.
10. Starr, P., and Liebhold-Schueck, R.: A theory of thyroid hormone action derived from the differences in the effect of sodium levo-thyroxine, sodium dextro-thyroxine, tri-iodothyronine, and potassium iodide on the uptake of radioactive iodine by the thyroid gland of normal human subjects, *Tr. A. Am. Physicians* **46**: 97–113, 1953.

THE EFFECTS OF EPINEPHRINE ON THYROXINE METABOLISM: CHROMATOGRAPHIC STUDIES OF SERUM AND TISSUE EXTRACTS*

BURTON KALLMAN, PH.D. AND PAUL STARR, M.D.
University of Southern California School of Medicine, Los Angeles 33, California

ABSTRACT

Labeled thyroxine was administered to humans and rats with and without the simultaneous administration of epinephrine. Epinephrine produced a decrease in the level of serum protein-bound iodine in humans. Chromatographic analysis of butanol extracts of serum and tissue was performed. Metabolites of thyroxine accumulated in the serum of some control subjects but not in the serum of those given epinephrine. Epinephrine did not produce an increase in thyroxine-derived triiodothyronine during the time in which it exerts a calorigenic effect. The data do not support the hypothesis that thyroxine must be deiodinated to triiodothyronine prior to entry into the peripheral tissues or during the course of hormonal activity.

RECENT work on the metabolism, distribution and excretion of thyroxine (Tx) has been interpreted as meaning that this tetraiodinated compound may not be a peripherally active thyroid hormone. It has been postulated that deiodination to 3,5,3′-triiodothyronine (TrIT) is required for entry into the peripheral cells and subsequent hormonal activity (1, 2). This hypothesis is based, in part, on the greater *in vivo* activity of TrIT and on the more similar distribution and excretion patterns of labeled TrIT and endogenously labeled thyroid hormone (3, 4). It has further been shown that thyroxine may be deiodinated both *in vivo* and *in vitro* to triiodothyronine (5–7), although Barker found this to be a variable phenomenon *in vitro* (8).

The present work was designed to test this hypothesis of mandatory deiodination of thyroxine to TrIT, using conditions which increase the utilization of thyroid hormone. Such conditions, by increasing the peripheral requirement for thyroid hormone, would presumably produce an increased rate of conversion of thyroxine to TrIT. Since thyroid hormonal action is probably catalytic and not associated with stoichiometric breakdown of the active material, it might be expected that this increased conversion would be reflected by an increased concentration of thyroxine-derived TrIT.

Received August 21, 1958.
* Supported by U. S. Public Health Service Grant #A-674.

In this study, epinephrine was utilized as an agent which increases the demand for thyroid hormone. The calorigenic effect of epinephrine has been shown to require the presence of thyroid hormone (9). In addition, epinephrine lowers the level of serum protein-bound iodine (10, 11) and increases the urinary excretion of iodide-I^{131} after the administration of labeled thyronines (12). Further evidence of an interrelation between epinephrine (and norepinephrine) and thyroid hormone is contained in the report of Brewster *et al.* (13) demonstrating that the effects of increased amounts of thyroid hormone are abolished in dogs with total sympathetic block.

MATERIALS AND METHODS

Thyroxine I^{131}. *l*-Thyroxine labeled with I^{131} in the 3′ or 5′ position was purchased from Abbott Laboratories, Oak Ridge, Tennessee. This material was chromatographically pure and was found to remain so for about a week. Labeled *l*-triiodothyronine was also purchased for R_f determinations and was found to be less stable than the isotopic thyroxine.

Butanol extraction. Serum samples were adjusted to pH3 with dilute hydrochloric acid and were extracted three times with approximately double volumes of *n*-butanol. After centrifugation, the butanol layers were pipetted off and pooled. The extracts were then taken to near dryness by vacuum distillation at 55–60° C. under nitrogen.

In the animal experiments, tissues were removed immediately after the animals had been killed by exsanguination under light ether anesthesia. These tissues were then homogenized in 15 ml. of distilled water in a Servall "Omnimixer" homogenizer immersed in an ice bath. The tissue samples consisted of both kidneys, both submaxillary glands, the entire heart, most of the liver and as much skeletal muscle as could be removed from one hind leg. Butanol extractions were performed in the same manner as with serum.

The concentrated extracts, especially those of liver and kidney, were viscous and highly pigmented. The amounts which could be placed on the chromatographic strips were limited to the order of 20 μl. by a tendency to overload the paper and spread from the line of origin.

Chromatographic methods. One-dimensional ascending chromatography was performed on Whatman 3-mm. filter paper. Tertiary amyl alcohol saturated with $2N$ NH_3 was usually used as the developing solvent; butanol-dioxane-ammonia was also used. Much variability in R_f values was noticed with both these solvent systems, but the values were constant within each chromatographic run. Both systems afforded good separation of thyroxine and TrIT, but the amyl alcohol system, in our hands, did not resolve iodide and thyroxine. All solvents were distilled immediately before use.

Unlabeled thyroxine, TrIT, iodide and mono- and diiodotyrosine[1] were also used as chromatographic markers. The location of the iodinated amino acids was determined by color development with Ninhydrin, diazotized sulfanilic acid or arsenious acid-ceric ammonium sulfate. Iodide was located by the black color formed with palladium chloride. These markers were not added to the experimental butanol extracts due to the aforementioned tendency to overload the paper. Several strips of the marker substances were run with each group of experimental materials.

[1] Thyroxine donated by Travenol Laboratories, Inc., Morton Grove, Ill.

Detection of radioactivity. Several instruments were used for the detection of radioactivity on the chromatograms. The strips were cut into 1-cm. segments which were counted individually in one of three detectors. A well-type scintillation counter, an end-window Geiger tube and an automatic sample-changing machine with a gas flow tube, were all used in order to analyze rapidly the large number of strips chromatographed in each experiment.

Recovery experiments. In experiments designed to determine if small amounts of TrIT could be detected in the presence of thyroxine, mixtures of the labeled materials were injected intraperitoneally into rats. The animals were killed at two hours and various tissues analyzed. Distinct triiodothyronine peaks were found in all the tissues studied. In a rat given 60 μc. of thyroxine and 1 μc. of TrIT, the recovery ratios for Tx:TrIT were—skeletal muscle, 12.5; heart, 22.7; serum, 24.7; kidney, 44.0; and liver, 65.0. In other studies, mixtures were given intravenously to athyrotic humans. The minimal amount of triiodothyronine injected was 10 μc. When this amount was given with 240 μc. of thyroxine, TrIT was found only as a shoulder on the thyroxine peak. When 20 μc. of TrIT was given with 240 μc. of thyroxine, a discrete TrIT peak was found in early blood samples.

RESULTS

Human subjects

Eight patients from the Thyroid Clinic of the Los Angeles County General Hospital, in various thyroidal states, were given intravenous I^{131}-labeled thyroxine. Essentially pure thyroxine was found in blood samples drawn at various time intervals from 4 of these patients. In a fifth case a small amount of TrIT and considerable activity at the solvent front were found in one sample. More complex patterns were obtained with the serum of the other 3 patients. TrIT was found present in varying amounts in these samples. Patient 6, who had a previous history of hepatic pathology, was studied on a second occasion. Only a small amount of TrIT was detected (butanol-dioxane chromatography). An additional patient with hepatic dysfunction, but presumably euthyroid, was also studied. Four early blood samples contained considerable TrIT as identified by amyl alcohol development.

The results of these studies are listed in Table 1, expressed as the percentage of the total radioactivity found on the chromatogram as thyroxine, TrIT or solvent-front activity. Peaks of activity detected at other locations have been included in the table in a position relative to their place on the chromatogram, *e.g.*, between thyroxine and TrIT.

The effects of epinephrine were studied in 6 other subjects in a total of 9 observations. Varying amounts of epinephrine[2] were administered intramuscularly. The physiologic effects were followed by measurements of the pulse rate and blood pressure, the patient's subjective opinions and, in some cases, by short periods of breathing through a basal metabolic rate

[2] Adrenalin in oil, 1:500, Parke Davis & Company.

TABLE 1. CHROMATOGRAPHIC ANALYSIS OF HUMAN SERUM AFTER INTRAVENOUS INJECTION OF THYROXINE-I^{131}

TABLE 1. CHROMATOGRAPHIC ANALYSIS OF HUMAN SERUM AFTER INTRAVENOUS INJECTION OF THYROXINE-I^{131}

Patient No.	Condition	Dose of I^{131} and thyroxine	Time after injection	Chromatographic components (% of radioactivity recovered)											
				Butanol-Dioxane-NH_3						Tert. amyl alc.-NH_3					
					Tx*		TrIT*		Solvent front		Tx		TrIT		Solvent front
1	Athyrotic; euthyroid	260 μc.; 7 μg.	3, 7, 24 hrs.		~100										
2	Myxedema	340 μc.; 15 μg.	3.5, 24 hrs.		~100										
3	Hyperthyroid	340 μc.; 15 μg.	3.5, 24 hrs.		~100										
4	Athyrotic; euthyroid	240 μc.; 11 μg.	20 mins.								94.0				1.8
			81 mins.								~100				
			2 hrs.								~100				
5	Myxedema; tuberculosis	200 μc.; 11 μg.	42 mins.		~100						~100				
			2.5 hrs.		~100						84.6				6.4
			6.5 hrs.		~100						~100				
			9.0 hrs.		54.3		2.9		19.7		40.0		5.3		15.7
			24.5 hrs.		~100						~100				
6	Athyrotic; hypothyroid	95 μc.; 6 μg.	30 mins.		50.5		5.5		23.9	15.1	27.1	21.7	5.5	12.8	15.9
	Biliary pathology		18.5 hrs.		32.6		16.3		28.1	9.8	29.4	18.8	4.5	9.2	28.2
6a		150 μc.; 9 μg.	30 mins.		87.7				2.9						
			2.5 hrs.		92.0				2.0						
			4.75 hrs.		72.1		2.0		4.9						
7	Hyperthyroid	200 μc.; 13 μg.	20 mins.		67.7				15.6		56.6	3.4	4.4		28.4
			1.25 hrs.	3.8	74.0				6.4		44.5				16.3
			3.23 hrs.	8.8	57.3				16.3						
			5.25 hrs.		65.5		3.9		10.7	6.1	16.1	6.0	1.4	3.4	49.2
8	Athyrotic; hypothyroid	200 μc.; 13 μg.	40 mins.		~100					14.7	38.8	24.3	1.8		12.8
			1.67 hrs.		~100					6.5,11.6	65.3		3.0		3.6
			3.67 hrs.		~100					14.7,22.1	50.0		2.0		4.8
			5.67 hrs.		~100					4.6,27.2	65.9		5.1		12.1
9	Jaundice: gastro-intest. ca.	120 μc.; 9 μg.	30 mins.		~100						86.7				2.3
			2.5 hrs.		~100					1.8	86.5				1.5
			4.75 hrs.		~100						86.7				
			24 hrs.	6.1	57.0			14.8	5.0	8.9–13.9	38.9		11.0		11.0

* Tx = Thyroxine. TrIT = Triiodothyronine.

TABLE 2. EFFECT OF INTRAMUSCULAR EPINEPHRINE ON THE LEVEL OF SERUM PROTEIN-BOUND IODINE (PBI)

Patient No.	Dose of epinephrine (mg., i.m.)	Time after epinephrine (mins.)	Serum PBI (μg./100 ml.)	Change (%)	Serum I^- (μg./100 ml.)	Patient No.	Dose of epinephrine (mg., i.m.)	Time after epinephrine (mins.)	Serum PBI (μg./100 ml.)	Change (%)	Serum I^- (μg./100 ml.)
11	0.5	3	7.6		2	13a	1.0 (twice)	0	3.4		4
		34	8.0	+ 5	4			11	2.4	−29	6
		78	7.5	− 1	3			36	1.8	−47	4
		98	6.9	− 9	3			57, 11	2.0	−41	6
12	0.5	1	7.3		4			88, 42	2.0	−41	7
		33	8.5	+15	4	14	1.0	0	7.0		>30
		71	6.7	− 8	3			35	8.0	+14	>60
		18	6.1	−16	4			105	8.2	+17	>30
2a	0.5 (twice)	0	6.6		8	15	1.0	0	4.2		>30
		84, 78	5.8	−12	10		0.4	15	4.6	+10	>30
		133, 127	5.4	−18	8		0.3	105, 75, 48	4.6	+10	>30
13	0.5 (twice)	0	7.8		8						
		19, 13	6.0	−23	10						
		84, 78	5.8	−36	8						

machine. An increase in metabolic rate was observed in 5 of the 7 tests in which this method was used. In addition, serial protein-bound iodine (PBI) and inorganic iodide determinations were obtained on the blood samples in 7 of these studies. These analyses were performed in the laboratory of the Thyroid Clinic. In 5 of the 7 cases studied, the expected decrease in the serum PBI level was observed. The results of these determinations are shown in Table 2.

Seven sera were found to contain only thyroxine and some solvent-front activity. In the remaining 2 sera there appeared to be small amounts of circulating TrIT (reaching a maximum of 4 per cent of the total activity); they also showed no decrease in PBI concentration, and a very high inorganic iodine level; these patients had the highest increase in oxygen consumption of the entire group. Chromatographic results are shown in Table 3.

Rat experiments

Eight male Long-Evans rats were given I^{131}-labeled thyroxine (intraperitoneally or intracardially) with and without epinephrine. Group I con-

TABLE 3. CHROMATOGRAPHIC ANALYSIS OF HUMAN SERUM AFTER INJECTION OF THYROXINE-I^{131} AND EPINEPHRINE

Patient No.	Dose of I^{131} and thyroxine (i.v.)	Dose of epinephrine (i.m.)	Response to epinephrine	Time after Tx	Chromatographic components (% of radioactivity recovered)			
					Tx	TrIT		Solvent front
10*	500 μc.; 29 μg.	0.3 mg. at 75 mins.	Pulse: 92→104	15 mins.	~100†			
			Subjective	80 mins.	~100			
				5.5 hrs.	~100			
11	528 μc.; 39 μg.	0.6 mg. at 25 mins.	Pulse: 68→100	24 mins.	~100†			
			Blood pressure: 130/80→140/66	42 mins.	~100			
			Subjective	4.3 hrs.	~100			
11a	200 μc.; 7 μg.	0.5 mg. at 6 mins.	Pulse: 64→80	9 mins.	71.9			15.0
			Oxygen uptake: no change	40 mins.	43.6			35.6
			Blood pressure: 120/62→138/80	84 mins.	85.4			4.1
			Subjective	104 mins.	70.4			8.2
12	200 μc.; 7 μg.	0.5 mg. at 5 mins.	Pulse: 80→100	6 mins.	73.4			10.9
			Oxygen uptake: no change	38 mins.	80.7			4.8
			Subjective	76 mins.	83.8			4.9
				103 mins.	84.3			3.0
12a	240 μc.; 6 μg.	0.5 mg. at 16 and 22 mins.	Pulse: 84→120	7 mins.	79.2			13.9
			Blood pressure: 110/70→160/80	43 mins.	65.4			21.8
			Oxygen uptake: increased	100 mins.	81.4			1.8
				149 mins.	84.0			2.3
13	240 μc.; 6 μg.	0.5 mg. at 10 and 16 mins.	Pulse: 88→96	8 mins.	92.1			2.4
			Blood pressure: 120/70→130/60	29 mins.	92.3			1.7
			Oxygen uptake: increased	94 mins.	92.2			2.1
				165 mins.	88.3			2.7
13a	236 μc.; 11 μg.	1.0 mg. at 26 and 72 mins.	Pulse: 70→98	22 mins.	89.6			3.2
			Blood pressure: 112/68→132/60	37 mins.	90.4			2.6
			Oxygen uptake: increased	62 mins.	89.3			2.5
				83 mins.	89.9			1.6
				114 mins.	89.9			2.2
14	250 μc.; 18 μg.	1.0 mg. at 36 mins.	Blood pressure: 156/110→163/90	22 mins.	76.8	2.6		2.7
			Oxygen uptake: increased	71 mins.	74.4	3.5		7.6
				141 mins.	75.1	3.9		2.2
15	250 μc.; 18 μg.	1.0 mg. at 34 mins.	Pulse 78→104	24 mins.	sample	lost		
			Blood pressure: 111/61→168/90	49 mins.	82.6	2.5		
		0.4 mg. at 66 mins.	Oxygen uptake: increased	141 mins.	65.9	3.8		
		0.3 mg. at 93 mins.	Subjective					

* Thyroid cancer patient.
† Analyzed with butanol-dioxane system; all other samples analyzed with tert. Amyl alcohol system.

TABLE 4. CHROMATOGRAPHIC ANALYSIS* OF GROUP I RAT TISSUES FOLLOWING INTRAPERITONEAL ADMINISTRATION OF *l*-THYROXINE-I^{131}

Tissue	Rat 1 of Group I (2 hrs.)			Rat 2 of Group I (5 hrs.)		
	% Radioactivity recovered			% Radioactivity recovered		
	Tx	TrIT	Solvent front	Tx	TrIT	Solvent front
Serum	61	2	4	77	1	2
Skeletal muscle	36	5	20	36	9	13
Heart	39	6		36	9	17
Salivary gland	52	9	13	92	3	
Liver	poor resolution			82	4	9
Kidney	lost			83	10	

* Tertiary amyl alcohol system.

tained 2 normal animals given thyroxine intraperitoneally (65 μc., 4 μg.) and killed at two and five hours, respectively.

The remainder of the animals had been thyroidectomized by two treatments with 500 μc. of I^{131}-iodide. Subsequent histologic sections showed no thyroid tissue in the necks or chests of these animals. All thyroidectomized animals except 2 in Group III were given 8 μg. of *l*-thyroxine daily by intraperitoneal injection for two weeks prior to their use.

Group II consisted of 2 rats which were given 80 μg. of epinephrine intramuscularly, thirty minutes after the intraperitoneal administration of labeled thyroxine. These animals were killed at two and three hours, respectively.

Group III contained 4 rats, 2 of which were not maintained with thyroxine prior to the experiment. These animals were given epinephrine intramuscularly (80 μg. per 100 Gm. of body weight) immediately after the intracardial injection of I^{131}-labeled thyroxine. They were killed at forty-five minutes. A marked increase in blood glucose concentration was found. Similar treatment, without epinephrine, caused no increase in the blood glucose level in a control rat. The results of these experiments are tabulated in Tables 4–6.

DISCUSSION

These experimental results do not indicate an increased rate of TrIT formation from thyroxine under conditions which increase the peripheral requirement for thyroid hormone.

TABLE 5. CHROMATOGRAPHIC ANALYSIS* OF GROUP II RAT TISSUES FOLLOWING ADMINISTRATION OF *l*-THYROXINE-I^{131} AND EPINEPHRINE

Tissue	Rat 1 of Group II (2 hrs.)			Rat 2 of Group II (5 hrs.)		
	% Radioactivity recovered			% Radioactivity recovered		
	Tx	TrIT	Solvent front	Tx	TrIT	Solvent front
Serum	53		15	43		28
Skeletal muscle	lost			46	7	12
Heart	62			68		7
Kidney	62		62	81		8

* Tertiary amyl alcohol system.

Circulating products of thyroxine metabolism appear to be reduced after administration of epinephrine to humans. Several of the human control sera had complex chromatographic patterns, showing TrIT and other metabolites of thyroxine. Essentially pure thyroxine was found in the serum of humans given epinephrine.

No evidence was found for an increase in thyroxine-derived TrIT in the tissues of epinephrine-treated rats. Small amounts of labeled TrIT were found in various tissues of both control and epinephrine-treated animals.

The large amounts of radioactivity found at the solvent front in many of the chromatograms did not appear to be artifacts. Studies were performed with beef kidney homogenates and I^{131}-labeled thyroxine and TrIT. These preparations were extracted, concentrated and chromatographed in the same manner as the experimental serum and tissue samples. The results were invariably "clean" chromatograms, with activity only in the thyroxine and TrIT areas. The addition of a reducing agent (thiouracil) to some of the experimental tissue homogenates or serum samples did not reduce the amount of solvent-front activity or the amount of activity located between the thyroxine and TrIT areas.

These results do not support the hypothesis that thyroxine must be deiodinated to TrIT prior to its entry into the peripheral cells or during the course of its metabolic activity.

Acknowledgments

The authors wish to acknowledge the technical assistance of Martha Kim and Pablo Villanueva. Protein-bound iodine determinations were performed by Maria Deering, and histologic work by Louis Cano.

TABLE 6. CHROMATOGRAPHIC ANALYSIS OF GROUP III RAT TISSUES FOLLOWING ADMINISTRATION OF *l*-THYROXINE-I^{131} AND EPINEPHRINE

A. *Rats Maintained with Thyroxine*

Tissue	Chromatographic system	Rat 1 of Group III % Radioactivity recovered						Rat 2 of Group III % Radioactivity recovered					
			Tx		TrIT		Solvent front		Tx		TrIT		Solvent front
Serum	tert. amyl		89						82		2		
	but.-diox.		81						82				
Skeletal Muscle	tert. amyl		71		2				33	6	7		7
	but.-diox.		95		3				77		2		
Heart	tert. amyl		50				10		54		1		14
	but.-diox.		44	36	7		8		40	17	3		10
Salivary gland	tert. amyl		34	10	7		35		43	11	12		
	but.-diox.		43		7		12		lost				
Liver	tert. amyl		69				5		52				
	but.-diox.		45	31					29	33	9		
Kidney	tert. amyl	24	59		5		4		69		6		5
	but.-diox.		78		7				45	19	12		8

B. *Rats Not Maintained with Thyroxine*

Tissue	Chromatographic system	Rat 3 of Group III % Radioactivity recovered						Rat 4 of Group III % Radioactivity recovered					
			Tx		TrIT		Solvent front		Tx		TrIT		Solvent front
Serum	tert. amyl		86						90		2		
	but.-diox.		80						73		4		
Skeletal muscle	tert. amyl		23	28			5	4	62		6		5
	but.-diox.	poor resolution						21	49		4		
Heart	tert. amyl	low activity							66		1		24
	but.-diox.		13	16	3		21	24	22		3		21
Salivary gland	tert. amyl		60		7		9	3	31		8		28
	but.-diox.		62		5		11		low activity				
Liver	tert. amyl		73		2		2		41		2		
	but.-diox.	7	59	25	4		4		poor resolution				
Kidney	tert. amyl		54		2		4	7	64		2		8
	but.-diox.		20	17	2		38	38	37		4		14

REFERENCES

1. Gross, J., and Pitt-Rivers, R.: Thyroid hormone physiology and biochemistry; triiodothyronine in relation to thyroid physiology, *Recent Progr. Hormone Res.* **10**: 109, 1954.
2. Gross, J.: The distribution of radioactive thyroid hormone in tissues, *in* The Thyroid. Brookhaven Symposia in Biology No. 7. Upton, N. Y., Brookhaven National Laboratory, 1954, p. 102.
3. Albert, A.; Ford, E., and Lorenz, N.: Effects of altered thyroid function upon the metabolism of endogenously labeled thyroid hormone, *Endocrinology* **53**: 50, 1953.
4. Keating, F. R., Jr., and Albert A.: Distribution and metabolism of radioactive *l*-triiodothyronine compared with radioactive *l*-thyroxine in the rat, (abstract) *J. Clin. Invest.* **32**: 580, 1953.
5. Pitt-Rivers, R.; Stanbury, J. B., and Rapp, B.: Conversion of thyroxine to 3,5,3′-triiodothyronine *in vivo*, *J. Clin. Endocrinol. & Metab.* **15**: 616, 1955.
6. Kalant, H.; Lee, R., and Sellers, E. A.: Metabolic fate of radioactive thyroid hormones in normal and propylthiouracil-treated rats, *Endocrinology* **56**: 127, 1955.
7. Albright, E. C.; Larson, F. C., and Tust, R. H.: *In vitro* conversion of thyroxin to triiodothyronine by kidney slices, *Proc. Soc. Exper. Biol. & Med.* **86**: 137, 1954.
8. Barker, S. B.: Cellular actions of thyroxine and similar compounds, *in* Regulation and Mode of Action of Thyroid Hormones. Vol. X of Ciba Foundation Colloquia on Endocrinology, ed. by G. Wolstenholme and E. Millar. Boston; Little, Brown and Company, 1957, p. 253.
9. Swanson, H. E.: Interrelations between thyroxin and adrenalin in the regulation of oxygen consumption in the albino rat, *Endocrinology* **59**: 217, 1956.
10. Williams, R. H.; Jaffe, H., and Kemp, C.: Effect of severe stress upon thyroid function, *Am. J. Physiol.* **159**: 291, 1949.
11. Eskelson, C. D.; Firschein, H. E., and Jensen, H.: Effect of epinephrine on thyroid hormone level in blood, *Proc. Soc. Exper. Biol. & Med.* **85**: 637, 1954.
12. Kallman, B., and Starr, P.: The effects of epinephrine on thyroxine metabolism: iodine excretion after various thyronine derivatives, *Endocrinology* **64**: 703, 1959.
13. Brewster, W. V., Jr.; Isaacs, J. P.; Osgood, P. F., and King, T. L.: The hemodynamic and metabolic interrelationships in the activity of epinephrine, norepinephrine and the thyroid hormones, *Circulation* **13**: 1, 1956.

JCEM

POTENTIATION OF THE VASOCONSTRICTOR ACTION OF TOPICAL NOREPINEPHRINE ON THE HUMAN BULBAR CONJUNCTIVAL VESSELS AFTER TOPICAL APPLICATION OF CERTAIN ADRENOCORTICAL HORMONES

DONALD J. REIS, M.D.

The Department of Medicine (Neurology), New York Hospital-Cornell Medical Center, New York, N. Y.

ABSTRACT

Studies were undertaken to estimate whether adrenocortical steroids can directly potentiate the vasoconstrictor activity of norepinephrine in the intact human being and whether this property is shared equally by various adrenal steroids. Most of the 21 subjects were in good health; 5 had migraine. Under direct visualization with a slit-lamp microscope the bulbar conjunctival capillary beds were inspected for basal activity and measurement of the thresholds for vasoconstriction following topically administered serial dilutions of norepinephrine. A 2.5 per cent ophthalmic suspension of either cortisone, hydrocortisone, Δ^1-cortisone, compound A, compound S or DCA was then instilled into one eye and basal activity and norepinephrine thresholds estimated again fifteen minutes later. The other eye served as control. It was demonstrated that hydrocortisone and cortisone consistently induced a threefold increase in sensitivity to norepinephrine, and Δ^1-cortisone induced a tenfold increase. Compounds A and S, on the other hand, had no effect. DCA was active in 4 of 10 subjects, causing a seventeenfold increase in sensitivity, on the average; in 6 subjects, however, it had no effect. No alteration in thresholds was noted in the control eye, indicating a direct action of the steroid on the blood vessels. Only Δ^1-cortisone and DCA, when active, produced a change in the basal morphology of the vessels. It is concluded that certain adrenocortical steroids can potentiate the vasoconstrictor action of norepinephrine, and that this activity does not appear to parallel the carbohydrate-regulating or salt-retaining effects of the steroid, but exists as an independent process.

INTRODUCTION

IT HAS been suggested that adrenocortical steroids may support vascular tone by potentiating the pressor action of norepinephrine (1). Increased sensitivity of blood vessels to topical norepinephrine after pretreatment with systemic cortisone or adrenocorticotropin (ACTH) has been suggested by the findings on direct visualization of the terminal vas-

Received May 20, 1959.

cular bed in the rabbit ear (2), and demonstrated in the mesoappendix of the adrenalectomized rat (1, 3). Inferentially this hypothesis has been supported by the augmentation of the pressor response to intravenous norepinephrine in the adrenalectomized dog (4), and in human subjects given large doses of cortisone (5) or desoxycorticosterone acetate (DCA) (6).

It is not known, however, whether the adrenocortical steroids have a direct potentiating action on norepinephrine with regard to constriction of the blood vessels in the intact human subject. Furthermore it has not been established whether this property, if present, is shared equally by all corticosteroids. In order to determine whether corticosteroids directly potentiate the action of norepinephrine on the terminal vascular bed of the normal human subject and whether differences exist between various steroids in this respect, the following studies were undertaken.[1]

METHOD

Fifty-one studies were carried out on 21 subjects. Five of the 21 were women, 4 of whom suffered from migraine headaches. One man had multiple sclerosis and another had migraine headaches. The remaining 14 subjects were healthy males between the ages of 22 and 30.

Observations were made on the bulbar conjunctival vessels with a Posar slit-lamp microscope at a magnification of 47×. The technique is described in detail by Lee (7) and by Ostfeld, Reis and Wolff (8). At this magnification one can readily identify arterioles, metarterioles, precapillary sphincters, capillaries, venules and arteriovenous shunts. The activity of the bulbar vessels in each eye was noted prior to each test. Intermittency of arteriolar flow (vasomotion), degree of arteriolar constriction, and number and patency of capillaries were evaluated and graded from 1+ to 4+ in ascending order of severity, with complete metarteriolar and precapillary sphincter spasm and blanched capillary beds representing a 4+ state. During the course of each observation a given vascular area was identified in each eye and revisualized with each subsequent observation in order to eliminate local variations within the expanse of the conjuctival field.

The sensitivity of precapillary sphincters and metarterioles to serial dilutions of norepinephrine in a phosphate buffer adjusted to a pH of 7.4 was next determined in both eyes by the direct instillation of 0.4 ml. of this substance into the conjunctival sac. Dilutions of norepinephrine were applied every three minutes, progressing from the weakest (1 part norepinephrine in 800,000 parts buffered base) to the most potent (1:10,000) until an end-point was reached. The end-point was defined as that concentration of norepinephrine which induced precapillary and metarteriolar spasm and resulting blanching of a given capillary bed (4+response). This end-point was reproducible independently by other observers, and once determined, could be reproduced by a single application of the effective concentration of norepinephrine, ruling out any additive effects of serial testing. The response to norepinephrine was immediate, lasted about ninety seconds, and did not weaken during the course of a particular study.

After initial observations of vascular activity and norepinephrine thresholds were made, 0.4 ml. of a suspension of one of the adrenocortical hormones was introduced into

[1] The steroids used in this study were generously provided by the Schering Corporation and Merck & Company, Inc.

the bulbar conjuctival sac of one eye. The hormones used were: cortisone (compound E), hydrocortisone (compound F), 11-desoxycorticosterone acetate (DCA), 11-dehydrocorticosterone (compound A), and 17-hydroxy-11-desoxycorticosterone (compound S), all of which were prepared in a 2.5 per cent suspension in a buffered phosphate ophthalmic solution. Δ^1-Cortisone (prednisone) was prepared as a 2.5 per cent suspension in sterile water. Observations were made in both the treated and untreated eye fifteen minutes after the steroids were introduced and any changes in appearance, behavior, and threshold response to topical norepinephrine were noted.

RESULTS

The results of these observations are summarized in Table 1, which shows the concentrations of topical norepinephrine necessary to produce vasoconstriction of the bulbar conjunctival vessels before and after pretreatment of the vascular bed by local instillation of various corticosteroids. The average increase in vascular sensitivity induced by each of the 6 steroids tested is also listed. At no time did the sensitivity of the vessels in the control (untreated) eye change during the course of an observation, indicating that any effect of a given steroid was an entirely local phenomenon and not secondary to systemic changes.

Both Δ^1-cortisone and DCA, when active, had an independent constrictive action on the bulbar conjunctival vessels. Δ^1-Cortisone caused a perceptible blanching of the capillaries, an increase in vasomotion, and an increase in arteriolar constriction. This effect was most marked at half an hour after application, whereas thresholds for norepinephrine changed within fifteen minutes—well before the steroid-induced constriction was observed.

Topical hydrocortisone, cortisone, and Δ^1-cortisone consistently increased vascular sensitivity to topical norepinephrine. Hydrocortisone and cortisone were about equally potent and induced a threefold lowering of the threshold on the average. Δ^1-Cortisone increased sensitivity, on the average, by a factor of 10. This is a statistically significant difference between Δ^1-cortisone and cortisone or hydrocortisone at the 1 per cent level of confidence.

Desoxycorticosterone acetate was entirely unpredictable in its activity. When active, it was capable of markedly depressing norepinephrine thresholds (average of seventeenfold in 4 subjects). On the other hand, 6 subjects demonstrated no alteration in the vascular response to norepinephrine after the application of DCA, even when tested four hours later.[2]

The data in Table 2 show that the ability of hydrocortisone to alter consistently the vascular thresholds to topical norepinephrine in 7 subjects was

[2] This would appear to be an "all or none" phenomenon. As such, it justifies estimation of the relative potency of DCA on the basis of the average of the positive results rather than on the average of all results.

TABLE 1. CHANGE IN CONCENTRATION* OF TOPICAL NOREPINEPHRINE (NE) NECESSARY FOR VASOCONSTRICTION OF BULBAR CONJUNCTIVAL VESSELS AFTER LOCAL INSTILLATION OF CERTAIN CORTICOSTEROIDS INTO THE

TABLE 1. CHANGE IN CONCENTRATION* OF TOPICAL NOREPINEPHRINE (NE) NECESSARY FOR VASOCONSTRICTION OF BULBAR CONJUNCTIVAL VESSELS AFTER LOCAL INSTILLATION OF CERTAIN CORTICOSTEROIDS INTO THE BULBAR CONJUNCTIVAL SAC

Dehydrocorticosterone (compound A)			Cortisone (compound E)			Hydrocortisone (compound F)			Desoxycorticosterone acetate (DCA)			Δ^1-Cortisone (prednisone)			Hydroxydesoxycorticosterone (compound S)		
Conc. of NE before steroid	Conc. of NE after steroid	$\Delta\left(\frac{\text{after}}{\text{before}}\right)$	Conc. of NE before steroid	Conc. of NE after steroid	$\Delta\left(\frac{\text{after}}{\text{before}}\right)$	Conc. of NE before steroid	Conc. of NE after steroid	$\Delta\left(\frac{\text{after}}{\text{before}}\right)$	Conc. of NE before steroid	Conc. of NE after steroid	$\Delta\left(\frac{\text{after}}{\text{before}}\right)$	Conc. of NE before steroid	Conc. of NE after steroid	$\Delta\left(\frac{\text{after}}{\text{before}}\right)$	Conc. of NE before steroid	Conc. of NE after steroid	$\Delta\left(\frac{\text{after}}{\text{before}}\right)$
1:25*	1:25	1	1:25	1:100	4	1:150	1:200	1.3	1:50	1:800	16	1:100	1:800	8	1:25	1:25	1
1:25	1:25	1	1:50	1:400	8	1:50	1:150	3	1:25	1:25	1	1:100	1:600	6	1:50	1:50	1
1:25	1:25	1	1:10	1:25	2.5	1:100	1:300	3	1:25	1:25	1	1:25	1:150	6	1:25	1:25	1
1:37.5	1:37.5	1	1:25	1:37.5	1:5	1:50	1:100	2	1:25	1:400	16	1:50	1:600	12	1:25	1:25	1
1:50	1:50	1	1:50	1:200	4	1:50	1:200	4	1:25	1:25	1	1:25	1:400	16	1:25	1:25	1
1:25	1:25	1	1:150	1:600	4	1:25	1:100	4	1:25	1:600	24	1:50	1:600	12	1:25	1:25	1
1:50	1:50	1	1:150	1:300	2	1:50	1:100	2	1:25	1:25	1	1:25	1:200	8	1:50	1:50	1
1:50	1:50	1	1:50	1:150	3	1:25	1:100	4	1:50	1:50	1	1:50	1:400	8	1:50	1:50	1
			1:100	1:200	2	1:50	1:100	2	1:50	1:50	1	1:25	1:600	16			
			1:50	1:100	2	1:100	1:200	2	1:25	1:300	12						
			1:50	1:100	2	1:25	1:100	4									
			1:25	1:100	4												
Average		*1*			*3.3*			*2.8*			*17.0*†			*10.2*			*1*

* Expressed as parts per thousand (*e.g.*, 1:25=1:25,000).
† Average of 4 positive results.

TABLE 3. COMPARISON OF CHANGE IN CONCENTRATION* OF TOPICAL NOREPINEPHRINE (NE) NECESSARY FOR VASOCONSTRICTION OF BULBAR CONJUNCTIVAL VESSELS IN 3 SUBJECTS AFTER PRETREATMENT WITH 6 DIFFERENT CORTICOSTEROIDS ON SEPARATE OCCASIONS

Subjects	Compound A			Cortisone			Hydrocortisone			DCA			Δ^1-Cortisone			Compound S		
	Conc. of NE before steroid	Conc. of NE after steroid	$\Delta\left(\frac{\text{after}}{\text{before}}\right)$	Conc. of NE before steroid	Conc. of NE after steroid	$\Delta\left(\frac{\text{after}}{\text{before}}\right)$	Conc. of NE before steroid	Conc. of NE after steroid	$\Delta\left(\frac{\text{after}}{\text{before}}\right)$	Conc. of NE before steroid	Conc. of NE after steroid	$\Delta\left(\frac{\text{after}}{\text{before}}\right)$	Conc. of NE before steroid	Conc. of NE after steroid	$\Delta\left(\frac{\text{after}}{\text{before}}\right)$	Conc. of NE before steroid	Conc. of NE after steroid	$\Delta\left(\frac{\text{after}}{\text{before}}\right)$
J.J.	1:25*	1:25	1	1:150	1:300	2	1:150	1:200	1.3	1:25	1:25	1	1:50	1:400	8	1:25	1:25	1
T.M.	1:25	1:25	1	1:50	1:100	2	1:50	1:100	2	1:25	1:600	24	1:25	1:400	16	1:25	1:25	1
B.L.	1:25	1:25	1	1:25	1:100	4	1:25	1:100	4	1:25	1:400	16	1:25	1:400	16	1:25	1:25	1

* Expressed as parts per thousand.

UNIVERSITY OF MICHIGAN LIBRARIES

TABLE 2. COMPARISON OF CHANGE IN CONCENTRATION* OF TOPICAL NOREPINEPHRINE (NE) NECESSARY FOR VASOCONSTRICTION OF BULBAR CONJUNCTIVE VESSELS IN 7 SUBJECTS AFTER PRETREATMENT WITH HYDROCORTISONE AND DCA ON SEPARATE OCCASIONS

Subject	Hydrocortisone			DCA		
	Conc. of NE before steroid	Conc. of NE after steroid	$\Delta\left(\frac{\text{after}}{\text{before}}\right)$	Conc. of NE before steroid	Conc. of NE after steroid	$\Delta\left(\frac{\text{after}}{\text{before}}\right)$
T.M.	1:50*	1:100	2	1:25	1:600	24
A.O.	1:50	1:200	4	1:25	1:25	1
E.W.	1:50	1:100	2	1:50	1:50	1
J.J.	1:150	1:200	1.5	1:25	1:25	1
B.C.	1:25	1:100	4	1:25	1:400	8
A.B.	1:100	1:200	2	1:50	1:50	1
H.F.	1:100	1:300	3	1:25	1:300	12

* Expressed as parts per thousand.

not predictably shared by DCA when this agent was applied to the same subjects on a different occasion.

Compounds A and S were consistently ineffectual in potentiating the effect of norepinephrine on bulbar conjunctival vessels, even up to four hours after application.

Individual variation in response was studied in 3 healthy male medical students (Table 3) receiving each of the 6 steroid preparations on different occasions. It may be seen that, with the exception of DCA, each corticosteroid produced similar reactions in all 3 subjects. Variations in baseline thresholds in a given subject from observation to observation were not unusual, and are consistent with data showing that individual thresholds may fluctuate from day to day (8). However, within the period of a given observation (usually less than one and a half hours), control thresholds did not change.

DISCUSSION

Site of action

These data demonstrate that certain adrenocortical steroids, when topically applied, are capable of sensitizing human bulbar conjunctival vessels to norepinephrine. This activity, when present, occurs within fifteen minutes after application of the steroid. That this effect is independent of any systemic action of these agents is demonstrated by the failure of the untreated eye to show any alteration in vascular reactivity while the vas-

cular sensitivity in the treated eye is maximal. Thus, alterations in systemic electrolyte distribution or changes in body fluid volume and distribution resulting from corticoid action, cannot explain this phenomenon. Except for Δ^1-cortisone, which was suspended in sterile water, the vehicles for both norepinephrine and the steroid were buffered to the pH of lacrimal secretion (tears) and isomolarity maintained. The vehicles themselves had no effect on the vessels observed. Doses of steroids, although greater than physiologic, were comparable. It can be assumed, therefore, that the changes in vascular reactivity observed were the result of the direct action of the steroids on the vascular bed of the bulbar conjunctiva.

It should be emphasized that the potentiation of the vasoconstrictor action of norepinephrine by adrenal steroids occurred in intact healthy subjects. This is in contrast with the observations that the potentiation of the vasoconstrictor activity of topical norepinephrine and epinephrine in the rat mesoappendix preparation after topical or parenteral administration of crude adrenal extract (1, 3) and the augmentation of the pressor response to infused norepinephrine in the dog pretreated with parenteral adrenal extract (4) occur only in the adrenalectomized animal. Our studies demonstrate that, in man, absence of the adrenals is not a prerequisite for the immediate potentiating effect of certain corticoids on norepinephrine constrictor activity in the bulbar conjunctival vessels.

Qualitative differences between corticoids

It is clear that important qualitative differences existed between the 6 corticoids tested, in regard to their capacity to potentiate the constrictor effect of topical norepinephrine on the human bulbar conjunctival vascular bed. Cortisone and hydrocortisone consistently diminished the thresholds threefold, and Δ^1-cortisone was equally consistent but three times more powerful than these other two agents. DCA was totally unpredictable in its activity. When effective, it was the most potent steroid tested; but more often than not, it was ineffective. This variability of response of human conjunctival vascular elements to potentiation of the action of norepinephrine by DCA was similar to the capricious and almost random behavior of this agent noted with regard to sodium chloride retention in healthy human subjects (9). Apparently other factors in the environment of the vascular bed are of importance in determining whether DCA will effectively potentiate norepinephrine in a given subject. Compounds A and S, steroids of less general potency, did not potentiate norepinephrine.

Similar studies utilizing direct visualization of the peripheral vascular bed in other species have yielded varied results. Parenteral or topical cortisone sensitizes the vessels of the adrenalectomized rat's mesentery to topical norepinephrine (1, 3). Results with the rabbit-ear preparation have

been equivocal (2, 10), and in the cheek pouch of the hamster (11, 12) cortisone appears to desensitize vessels to topical norepinephrine. Parenteral or topical DCA is consistently ineffective in sensitizing vessels to norepinephrine in the mesentery of the adrenalectomized rat (1, 3). The comparability of these studies with ours, however, is severely limited by differences in species, techniques, the particular vascular bed observed, the type and dosage of drug preparation, the mode of administration, and the presence or absence of anesthesia.

Relationship of vasoconstrictor potentiation to other corticoid functions

It does not appear that the qualitative differences that exist between the different corticoids examined in regard to their ability to potentiate topical norepinephrine in the bulbar conjunctival vascular bed parallels the relative activity of these agents in regard to salt retention or regulation of carbohydrate metabolism.

In Figure 1 the 6 compounds are graphically ranked in order of relative potency with reference to: (A) salt-retaining activity as measured by the excretion ratio of sodium/potassium in the rat; (B) glycogen deposition and muscle work in the rat; and (C) sensitization of human conjunctival vessels to topical norepinephrine. It may be seen that there is no parallelism between these functions. If it be postulated that the phenomenon of potentiating norepinephrine is related to the salt-retaining capacity of a given steroid, then one would expect the effect of DCA to be powerful and predictable and that of cortisone, hydrocortisone, and particularly Δ^1-cortisone to be less than demonstrated. On this basis, compounds S and A would be expected to be more active. If carbohydrate regulation of a corticoid were the determining factor, it would be expected that compounds A and S would be more active than they are, and DCA would consistently be ineffectual.

It is concluded, therefore, that the relative activity of these corticoids in potentiating the action of norepinephrine on human bulbar conjunctival vessels is an independent function which cannot be strictly correlated with the two classic major categories of adrenal activity, *viz*, carbohydrate regulation and salt retention.

Mode of action

It is not possible on the basis of these studies to state whether or not these corticoids have an independent vasoconstrictor function unrelated to catechol amine activity.

Williams, Heiple and Ebert (10) and Ashton and Cook (2), studying the peripheral vascular bed of the rabbit ear, noted that cortisone in large systemic doses was independently capable of constricting arterioles and

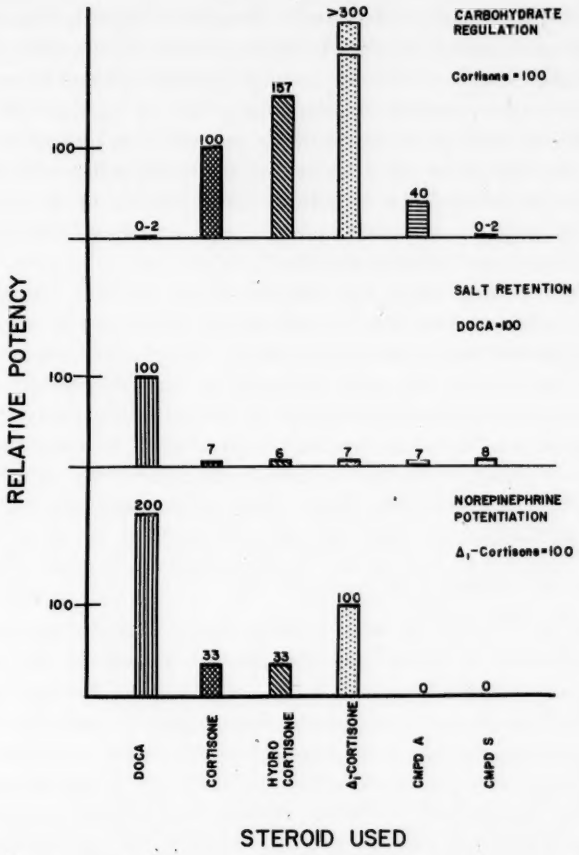


FIG. 1. Illustrating the relative potency of 6 adrenocortical steroids compared with regard to carbohydrate regulation and salt retention (as derived from Russell (13) and Thorn *et al.* (14)) and with regard to potentiation of norepinephrine. An arbitrary value of 100 is assigned to a particular steroid as indicated in the upper right-hand corner of each of the three compared groups. It should be noted that the assigned activity of desoxycorticosterone acetate with regard to norepinephrine potentiation is derived from only the positive responses (see text). These data demonstrate that the capacity of a steroid to potentiate norepinephrine does not predictably parallel that steroid's function with regard to carbohydrate regulation or salt retention.

blanching capillaries. However, these observations are at variance with our results. Cortisone and hydrocortisone, both moderate potentiators of norepinephrine in the human conjunctival bed, did not alter the resting configuration of these vessels whereas Δ^1-cortisone produced consistent perceptible changes in vasomotion, arteriolar constriction and capillary

filling within half an hour after application. It is possible that this difference may be only one of degree. As cortisone and hydrocortisone are one-third as effective as Δ^1-cortisone in potentiating topical norepinephrine, they may similarly produce proportionally less of an induced change in vascular caliber. This, perhaps, could be perceived in human conjunctival vessels only by the use of the greater magnification utilized in the study of rabbit-ear preparations. Nevertheless, the presence of small local concentrations of endogeneous norepinephrine cannot be excluded as the cause of a corticoid-induced constrictor effect.

These observations have not clearly ruled out the possibility that changes in vascular sensitivity after steroid application merely reflect changes in permeability of mesothelium and endothelium to topical norepinephrine. This would seem an unlikely explanation of the observed effects on the grounds that: (a) the norepinephrine molecule is small enough to allow almost free diffusion through body tissues, and (b) that Δ^1-cortisone and DCA produce vascular constriction before any constrictor agent is deposited in the same field. Direct evidence against this interpretation, however, is lacking.

Relationship to systemic blood pressure

Parenteral ACTH, DCA, and cortisone have been demonstrated under certain conditions to potentiate the pressor action of infused norepinephrine in the intact human (5, 6) or the adrenalectomized dog (4). The relevance of these observations to our data is highly suggestive but necessarily speculative. One must be guarded in extending conclusions derived from observations on the local alterations produced in bulbar conjunctival vessels under the limited conditions of this study to the complex and incompletely understood mechanisms involved in the maintenance of systemic blood pressure. It has been clearly demonstrated by Zweifach and Metz (15) that changes in the terminal vascular bed of the rat by pressor agents may vary with the region examined, and that there is no predictable correlation between changes in systemic blood pressure and the response of a given vascular bed to topically applied vasoactive principles (16). These authors emphasize the fact, "One cannot treat the peripheral vascular bed as an organ system. It has qualities which are reflections of local metabolic needs" (15).

Relevance to other organ systems

Our observations have demonstrated that vascular cells may be sensitized to the constrictive action of norepinephrine by previous application of certain adrenocortical steroids. However, the peripheral vascular bed of the human conjunctiva may be viewed as a cellular model having the special-

ized function of regulating blood flow but still sharing basic biologic properties with other body tissues. Indeed, there is extensive experimental evidence, reviewed recently by Ramey and Goldstein (17), that the products of the adrenal medulla and corticosteroids interact not only with regard to vascular regulation but in other vital metabolic functions as well.

The actions of various and ubiquitous steroid hormones on vascular tissue is not well understood. It has been shown that changes in the systemic endocrine environment may alter vascular reactivity in the human bulbar conjunctival bed. Landesman and coworkers, for example, have demonstrated consistent and significant changes in these vessels during the menstrual cycle (18) and uncomplicated pregnancy (19), and Lee has shown that the conjunctival vascular changes are marked in patients with Cushing's disease (20). No previous studies on the bulbar conjunctival vessels, however, have utilized the described technique of directly applying the agents under study to the vascular tissue of one eye in the intact human subject while utilizing the other eye as a control for systemic changes. Our experience with this method would indicate that such an approach to the study of vascular pharmacology is simple, direct, and presents a well controlled experimental system for investigation into this area of human biology.

REFERENCES

1. Fritz, I., and Levine, R.: Action of adrenal cortical steroids and norepinephrine on responses of stress in adrenalectomized rats, *Am. J. Physiol.* **165:** 456, 1951.
2. Ashton, N., and Cook, C.: *In vivo* observations of the effects of cortisone upon the blood vessels in rabbit ear chambers, *Brit. J. Exper. Path.* **33:** 445, 1952.
3. Zweifach, B. W.; Shorr, E., and Black, M. M.: Influence of the adrenal cortex on the behavior of the terminal vascular bed, *Ann. New York Acad. Sc.* **56:** 626, 1953.
4. Ramey, E. R.; Goldstein, M. S., and Levine, R.: Action of norepinephrine and adrenal cortical steroids on blood pressure and work performance in adrenalectomized dogs, *Am. J. Physiol.* **165:** 450, 1951.
5. Kurland, G. S., and Freedberg, A. S.: The potentiating effect of ACTH and of cortisone on pressor response to intravenous infusions of *l*-norepinephrine, *Proc. Soc. Exper. Biol. & Med.* **78:** 28, 1951.
6. Raab, W.; Humphreys, R. J., and Lepeschkin, E.: Potentiation of pressor effects of norepinephrine and epinephrine in man by desoxycorticosterone acetate, *J. Clin. Invest.* **29:** 1397, 1950.
7. Lee, R. F.: Anatomical and physiological aspects of the capillary bed in health and disease, *Angiology* **6:** 369, 1955.
8. Ostfeld, A.; Reis, D. J., and Wolff, H. G.: Studies on headache, *A.M.A. Arch. Neurol. & Psychiat.* **77:** 113, 1957.
9. Soffer, L. J.; Gabrilove, J. L., and Jacobs, M. D.: Further studies with the salt tolerance test in normal individuals and in patients with adrenal cortical hyperfunction, *J. Clin. Invest.* **28:** 1091, 1949.
10. Williams, C. D., Jr., Heiple, K. G., and Ebert, R. H.: The effect of cortisone on

vascular reactivity: *in vivo* observations using the rabbit ear chamber technique, *J. Lab. & Clin. Med.* **44:** 210, 1954.

11. WYMAN, L. C.; FULTON, G. P., and SHULMAN, M. H.: Effect of adrenal insufficiency and experimental hypercorticalism on the circulation in the hamster cheek pouch, *Ann. New York Acad. Sc.* **56:** 643, 1953.
12. SUGIERA, H.: The action of epinephrine on the blood vessels of cortisone treated animals, *Anat. Rec.* **118:** 561, 1954.
13. RUSSELL, J.: *in* Textbook of Physiology, ed. 17, J. F. Fulton, Editor. Philadelphia, W. B. Saunders Company, 1955, Chapter 60, p. 1173.
14. THORN, G. W.: RENOLD, A. E.; MORSEL, N. I.; GOLDFIEN, A., and REDDY, W. J.: Highly potent adrenal cortical steroids: structure and biologic activity, *Ann. Int. Med.* **43:** 979, 1955.
15. ZWEIFACH, B. W., and METZ., D. B.: Regional differences in response of terminal vascular bed to vasoactive agents, *Am. J. Physiol.* **182:** 155, 1955.
16. ZWEIFACH, B. W., and METZ., D. B.: The relation of blood-born agents acting on mesenteric vascular bed and general circulatory reactions, *J. Clin. Invest.* **34:**653, 1955.
17. RAMEY, E. R., and GOLDSTEIN, M. S.: The adrenal cortex and the sympathetic nervous system, *Physiol. Rev.* **37:** 155, 1957.
18. LANDESMAN, R.; DOUGLAS, R. G.; DREISHPOON, G., and HOLZE, E.: The vascular bed of the bulbar conjunctiva in the normal menstrual cycle, *Am. J. Obst. & Gynec.* **66:** 988, 1953.
19. LANDESMAN, R.; DOUGLAS, R. G.; DREISHPOON, B. A., and LEE, R. E.: The bulbar conjunctival vascular bed in normal pregnancy, *Am. J. Obst. & Gynec.* **65:** 876, 1953.
20. LEE, R. E.: Hemodynamic changes in the bulbar conjunctival capillary bed of subjects with hypertension associated with "Cushing's syndrome" or pheochromocytoma, *Am. J. Med.* **19:** 203, 1955.

STEROID PRODUCTION BY HUMAN ADRENAL ADENOMATA AND NONTUMOROUS ADRENAL TISSUE *IN VITRO**

RICHARD E. BAILEY, M.D., CHARLES I. SLADE, M.B., AMOS H. LIEBERMAN, M.D. AND JOHN A. LUETSCHER, JR., M.D.

Department of Medicine, Stanford University School of Medicine, San Francisco and Palo Alto, California

ABSTRACT

Corticosteroid production by adrenal tissue slices was studied *in vitro* by means of an incubation technique. Adenomata from patients with Conn's syndrome and nontumorous adrenal tissue from patients with idiopathic edema or gonadal dysgenesis produced cortisol, corticosterone and aldosterone. Hyperplastic adrenal tissue from patients with Cushing's syndrome produced cortisol, corticosterone and, in one instance, aldosterone. Although it was not possible to correlate any particular secretory pattern with the clinical and pathologic diagnosis in this small series of patients, the pattern of hormone production *in vitro* provided additional and independent information of interest and possible value in interpretation of the pathologic physiology of these cases.

THE measurement of steroid formation *in vitro* as a direct assessment of the functional capacity of adrenal tissue was proposed by Brady (1). Several investigators (2–11) have utilized incubation procedures for qualitative and quantitative estimation of steroid formation by human adrenal tissue. These methods circumvent some difficulties and uncertainties associated with the measurement of circulating hormone levels and urinary metabolites, and provide information about the source of production. The purpose of this paper is to present results obtained on incubation of adrenal tissue from 3 patients with Conn's syndrome ("primary aldosteronism," "mineralocorticoid excess") due to adrenal adenoma, 2 patients with Cushing's syndrome due to adrenal hyperplasia, 1 patient with idiopathic edema, and 1 patient with gonadal dysgenesis.

MATERIALS AND METHODS

The incubation medium was Krebs-Ringer bicarbonate buffer to which 200 mg. of glucose per 100 ml. was added.

Received July 31, 1959.

* This project was supported by a research grant (A-630) from the National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service.

At the time of surgery, adrenal tissue was placed in vacuum flasks containing 50 ml. of pre-frozen buffer and 50 ml. of liquid buffer. The tissue was quickly trimmed free of fat, weighed, and a representative portion taken for histologic examination. Slices approximately 0.5 mm. thick were made with a Stadie-Riggs hand microtome. A 4-ml. volume of buffer was added for each 100 mg. of incubated tissue. The weight of tissue per incubation flask ranged between 300 and 1,200 mg. Incubations were performed at 37.5° C. under 95 per cent oxygen and 5 per cent carbon dioxide in a Dubnoff metabolic shaker. After thirty minutes of pre-incubation, the medium was decanted. Fresh buffer was then added and incubation continued for three more hours. In most experiments, comparisons were made between steroid production in buffer, and in buffer containing serum (2.5 ml. of serum per 100 ml. of buffer) or corticotropin (Armour lyophilized bovine ACTH, 5 I.U. per 100 ml. of buffer). Serum was obtained from the patient several hours prior to surgery. The extraction of media and preliminary chloroform-formamide chromatography were performed as described by Mulrow *et al.* (12). Ultraviolet photoprinting of the chromatograms was effected by wrapping chromatogram and printing paper on a hand-rotated kymograph drum, and exposing briefly to a G.E. Sterilamp. The fraction containing aldosterone was eluted from the chromatogram and measured by bioassay (13). The cortisol fractions were rechromatographed in the Bush C system, and corticosterone fractions were re-run in the Bush B-4 system. Cortisol and corticosterone were identified by relative mobilities in two chromatographic systems, by ultraviolet absorption curves, soda fluorescence, blue tetrazolium reduction and, in the adrenal incubates from the first 2 patients, by sulfuric-acid chromogens. Following elution, cortisol and corticosterone were quantitated by ultraviolet absorption and the color developed on reaction with blue tetrazolium.

Urinary 17-ketosteroids were measured by the method of Drekter *et al.* (14), urinary 17-hydroxycorticosteroids by a modification of the method of Silber and Porter (15), and aldosterone by extraction (16, extract B), chromatography (17), and bioassay (13).

CASE HISTORIES

Patient 1

H.K. was a 44-year-old white female who had hypertension for twelve years, without polyuria, muscle weakness or alkalosis. Hypokaliemia was discovered incidentally; potassium depletion was in the order of 350 mEq. Urinary pH was neutral, and the ammonia/titratable acidity ratio was high. The 24-hour excretions of urinary steroids were: 17-ketosteroids (17-KS), 4.0–12.6 mg.; 17-hydroxycorticosteroids (17-OH-CS), 5.6–10.7 mg.; and aldosterone, 38 μg. A benign adenoma weighing 2.1 Gm. was removed at operation, following which the blood pressure fell gradually and electrolyte abnormalities disappeared.

Patient 2

M.C. was a 33-year-old white female who had hypertension and severe headaches for five years. There was no evidence of muscle weakness, polyuria or alkalosis. The 24-hour excretions of urinary steroids were: 17-KS, 17.6–25.4 mg.; 17-OH-CS, 5.4–11.2 mg.; and aldosterone, 18 and 56 μg. A benign adenoma weighing 2.5 Gm. and a small portion of adjacent "normal" tissue were removed at operation. Remission of clinical and laboratory abnormalities followed.

Patient 3

L.L. was a 37-year-old white female who had hypertension, discovered four months prior to investigation. The blood pressure remained constant at about 180/120 mm.

Hg. Headaches had been frequent since childhood. Mild polyuria and polydipsia were present, but there was no muscle weakness. Potassium depletion was in the order of 300 mEq. The patient was in a state of alkalosis, and had dilute, neutral urine with a high ammonia/titratable acidity ratio. The 24-hour excretions of urinary steroids were: 17-KS, 8.9–10.2 mg.; 17-OH-CS, 4.5–9.1 mg.; and aldosterone, 18 and 26 μg. Removal of a benign adenoma weighing 0.75 Gm. and a small portion of adjacent "normal" tissue was followed by gradual reversion of the abnormalities to normal.

Patient 4

D.R.[1] was a 13-year-old white female with a two-year history of edema and idiopathic epilepsy. There was no history of polydipsia, polyuria or muscle weakness. Sodium restriction and diuretic therapy were partially successful in controlling the edema. Serum electrolyte levels had been normal prior to chlorothiazide therapy. The 24-hour urinary excretion of 17-KS was 7.2 mg. and of 17-OH-CS, 4.8 mg. At the time of surgery, a biopsy specimen was removed from the left adrenal, and bilateral wedge biopsy specimens were removed from the ovaries. The adrenal weighed 6 Gm. and was histologically normal. The wedge biopsy findings were diagnostic of polycystic ovaries. Unilateral adrenalectomy did not result in clinical improvement.

Patient 5

L.J. was a 23-year-old white female who was found to have primary amenorrhea, sexual infantilism, male sex chromatin pattern, various developmental anomalies, osteoporosis, diabetes and hypertension. The baseline 24-hour excretion of 17-KS was 3.3–6.7 mg. and of 17-OH-CS, 10.0–12.9 mg. After administration of 9α-fluorohydrocortisone (5 mg. per day by mouth, in fractional doses) for five days, the 24-hour excretion of 17-KS was 2.3 mg. and of 17-OH-CS, 2.1 mg. Following administration of corticotropin (40 I.U. of Armour ACTH-gel every twelve hours intramuscularly) for two days, the 24-hour excretion of 17-KS was 28.9 mg., and of 17-OH-CS, 107.5 mg. The titer of urinary gonadotropin was greater than 100 mouse units. A dysgenetic gonad with "hilus cells," and the right adrenal gland (4.8 Gm.) were removed at operation. The adrenal tissue was histologically normal.

Patient 6

O.M. was a 51-year-old white female who had spontaneous ecchymoses for three years. She was florid-faced, with central obesity and thin extremities. Her blood pressure varied between hypertensive (170/100) and normotensive (128/78) levels. Serum electrolyte levels were normal. The 24-hour urinary excretion of 17-KS was 23.9 mg. and of 17-OH-CS, 23.3 mg. Following administration of 9α-fluorohydrocortisone (16 mg. per day orally in fractional doses) for five days, the 24-hour excretion of 17-KS was 19.8 mg. and of 17-OH-CS, 18.0 mg. Following intramuscular administration of corticotropin (40 I.U. of Armour ACTH-gel every twelve hours) for three days the 24-hour excretion of 17-KS was 80.7 mg. and of 17-OH-CS, 194.0 mg. The left adrenal gland (12 Gm.) and 80 per cent (2.5 Gm.) of the right adrenal were removed at operation. The pathologist's diagnosis was adrenocortical hyperplasia.[2] Improvement of Cushing's syndrome followed surgery.

---

[1] Studied through the courtesy of Dr. Laurance W. Kinsell.

[2] Dr. Robert A. Jeffrey studied the tissue from these cases and kindly allowed the authors to quote his conclusions.

Patient 7

J.A. was a 17-year-old white male with typical Cushing's syndrome of four years' duration. Blood pressure was labile, but was usually normal. The baseline 24-hour excretion of urinary 17-KS was 14.9 mg. and of 17-OH-CS, 26.9 mg. These excretions increased following administration of ACTH (40 I.U. intramuscularly every twelve hours) for two days to 50.0 and 238.0 mg., respectively. Following administration of 9α-fluorohydrocortisone (5 mg. per day orally in fractional doses) for five days, the 24-hour excretion of 17-KS was 14.5 mg., and of 17-OH-CS, 10.3 mg. Total right adrenalectomy (6.8 Gm.) and 90 per cent subtotal left adrenalectomy (9.75 Gm.) was performed. The diagnosis was bilateral adrenocortical hyperplasia. The patient's condition improved after surgery.

RESULTS

Figures 1–6 show the result of incubations in terms of micrograms of steroid released per gram of tissue per hour. The values for cortisol (F) and corticosterone (B) were calculated from the measurements of ultraviolet-light absorption. Substantial agreement was obtained between these values and those calculated from color developed upon reaction with blue tetrazolium.

The results of incubation of the adrenal adenomata from Patients 1 and 2 (the mineralocorticoid syndrome) are shown in Figure 1. These adenomata secreted cortisol and corticosterone in considerably greater quantities than aldosterone when incubated with buffer alone. The addition of serum to the medium resulted in a large increase in the amounts of cortisol, cor-

FIG. 1. Steroid production *in vitro* by adenomatous adrenal tissue from Patients 1 and 2 (Conn's syndrome).

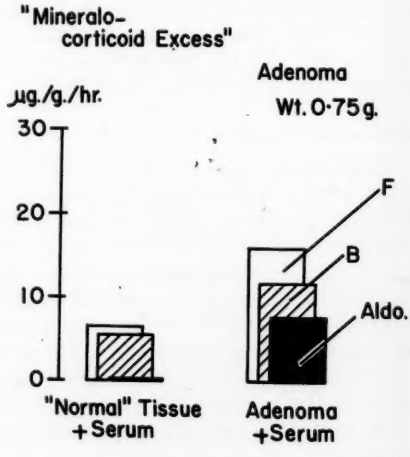


FIG. 2. Steroid production *in vitro* by adenomatous tissue and adjacent nontumorous adrenal tissue from Patient 3 (Conn's syndrome).

ticosterone and aldosterone recovered. In the second case, "normal" adrenal tissue adjacent to the adenoma was incubated. The quantities of cortisol, corticosterone and aldosterone recovered were considerably less than from the adenoma incubate.

The results of incubation of the adenoma and of the adjacent nontumorous adrenal tissue from Patient 3 (the mineralocorticoid syndrome) are shown in Figure 2. Cortisol and corticosterone were recovered in greater quantities than aldosterone. All 3 hormones were recovered in greater amounts from the adenoma incubates than from the "normal" tissue incubates.

In Figure 3 are shown the results of incubation of adrenal tissue from Patient 4 (idiopathic edema).[3] Cortisol, corticosterone and aldosterone

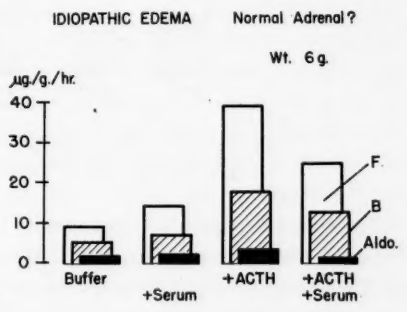


FIG. 3. Steroid production *in vitro* by adrenal tissue from Patient 4 (idiopathic edema).

were recovered from the incubation with buffer alone. The yield of all 3 hormones appeared to be slightly augmented by the addition of serum to the medium. The addition of ACTH caused a greater increase. No further increase was noted after incubation with both ACTH and serum added to the medium.

In Figure 4 are shown the results of incubation of adrenal tissue from Patient 5 (gonadal dysgenesis). Cortisol, corticosterone and aldosterone were recovered in similar quantities from the incubation with buffer alone and from incubation with buffer plus either the patient's serum or serum from a healthy female. The addition of ACTH resulted in a marked rise in cortisol output but little change in corticosterone and aldosterone production. When both ACTH and the patient's serum were added, a tenfold rise in cortisol secretion occurred, without any significant change in the secretion of either corticosterone or aldosterone.

In Figure 5 are shown the results of incubation of adrenal tissue from

[3] Steroid production in this case may have been influenced by the inadvertent use of ice-cold 0.154 *M* $NaHCO_3$, instead of buffer, as the carrying fluid between the operating room and the laboratory.

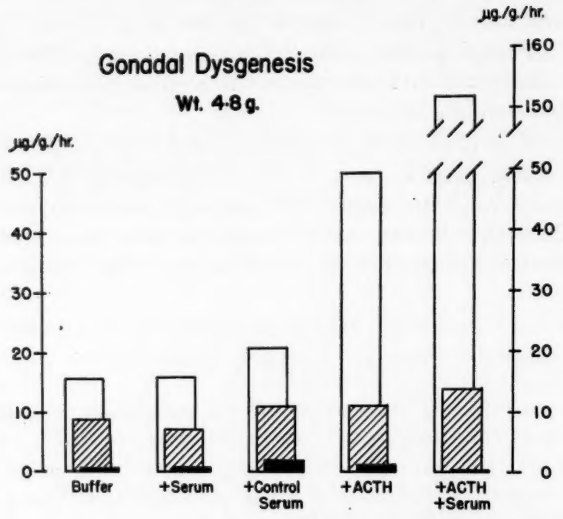


FIG. 4. Steroid production *in vitro* by adrenal tissue from Patient 5 (gonadal dysgenesis with diabetes and hypertension).

Patient 6 (Cushing's syndrome). Cortisol and corticosterone were recovered after incubation with buffer alone. The addition of the patient's serum had little or no effect. When ACTH was added, there was a large increase in the amounts of cortisol and corticosterone recovered. Aldosterone was not found in any of these incubates.

In Figure 6 are shown the results of incubation of adrenal tissue from

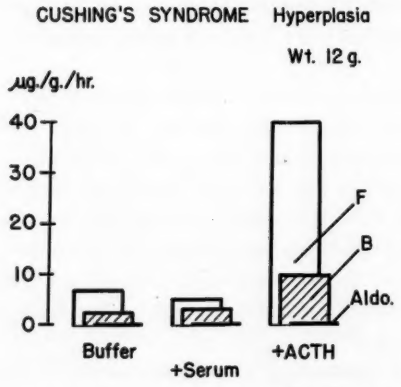


FIG. 5. Steroid production *in vitro* by adrenal tissue from Patient 6 (Cushing's syndrome).

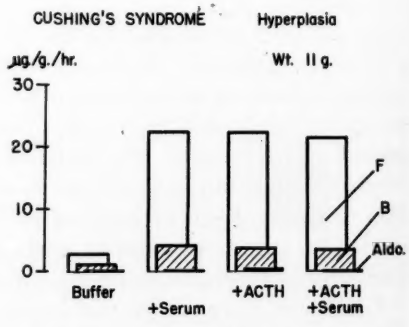


FIG. 6. Steroid production *in vitro* by adrenal tissue from Patient 7 (Cushing's syndrome).

Patient 7 (Cushing's syndrome). Incubation in buffer yielded cortisol and corticosterone. Incubation in buffer plus serum resulted in a large increase in cortisol and a substantial increase in corticosterone. A similar result was obtained when ACTH was added to the buffer. No additive effect of ACTH and serum was observed. Only when ACTH was added to the buffer was a measurable quantity of aldosterone found.

DISCUSSION

The hormones released from the surviving adrenal tissue studied in these experiments appeared in patterns generally resembling those secreted by normal tissue *in vivo*. Cortisol was found in the largest amounts in every case, accompanied by smaller amounts of corticosterone, and still less aldosterone—sometimes too little to detect.[4]

The steroid secreted *in vitro* by the tissue presumed to account for the various adrenal abnormalities described, was not limited to a single hormone in any case. Aldosterone appeared in appreciable quantities in the incubates of tumors from cases of Conn's "primary aldosteronism," but larger amounts of cortisol and corticosterone were also found. Substantial quantities of cortisol and corticosterone have been recovered from similar tumors by other investigators (4, 18, 19), and various interpretations have been put on the significance of these hormones (20, 21). An appreciable quantity of aldosterone was also secreted by tissue from the patient with idiopathic edema, offering some support for suggestions that aldosterone plays a role in this condition (22–24). Cortisol and corticosterone were also found in incubates of adrenals from cases of Cushing's syndrome, but the quantities did not differ greatly from those secreted by an equivalent weight of normal tissue. It would seem that the larger weight of hyperplastic tissue in Cushing's syndrome is a major factor in producing an excess of cortisol and corticosterone. Aldosterone, on the other hand, was not detected in control incubations of tissue from cases of Cushing's syndrome.

Some of these qualitative or quantitative differences might be attributed to the origin of the tissue from the glomerular zone, which normally produces aldosterone and corticosterone, or from the fascicular zone, in which production of cortisol predominates. However, it was not easy to define, identify or separate the normal zones in the abnormal tissues, especially in the adenomata, in which cells appeared in large nests. In cases of Cushing's syndrome, the extensive folding of the hyperplastic tissue made identification difficult, but the cells appeared to form strands resembling the fascicular zone. In the "normal" glands, a fair representation of both zones was probably present. The pattern of hormone production *in vitro* provides in-

[4] Other weaker bands of ultraviolet-absorbing material were noted but not identified in chromatograms of several incubates.

dependent information of interest and possible value in addition to the clinical and pathologic findings.

Although the production and release of hormones from surviving adrenal tissue continued for many hours after operation, observations were limited to the early hours before any severe decline in activity was evident. Nevertheless, calculations show that the secretion rate per gram of tissue *in vitro* was lower than that estimated to occur *in vivo*. The Krebs-Ringer bicarbonate buffer with glucose may have been inadequate; the addition of bovine corticotropin, however, produced a consistent and substantial increase in cortisol production. Corticosterone and aldosterone were usually found in increased amounts after ACTH was added. Since factors enhancing corticosteroid release from rat adrenals have been found in serum and urine (12, 25), the effect of the patient's serum was also studied. Although the results were variable, the addition of serum could enhance corticosteroid release as much as when ACTH was added. Mixtures of ACTH and serum sometimes appeared to have additive effects. More experience is required, however, before these results can be properly assessed.

Acknowledgment

The authors are greatly indebted to C. Armitage, A. Dowdy, J. Harvey, W. Lew, A. Nolan, L. Poo and C. Zazulak for their invaluable assistance.

REFERENCES

1. Brady, R. O.: A rapid method for the qualitative and quantitative estimation of the physiological activity of the adrenal cortex, *Endocrinology* **52**: 49, 1953.
2. Cooper, D. Y.; Roberts, J. M., and Touchstone, J. C.: Steroid production by incubated human adrenal tissue, *Surg. Forum* **5**: 583, 1954.
3. Heard, R. D. H.; Bligh, E. G.; Cann, M. C.; Jellinck, P. H.; O'Donnell, V. J.; Rao, B. G., and Webb, J. L.: Biogenesis of the sterols and steroid hormones, *Recent Progr. Hormone Res.* **12**: 45, 1956.
4. Milne, M. D.; Muehrcke, R. C., and Aird, I.: Primary aldosteronism, *Quart. J. Med.* (N. S.) **26**: 317, 1957.
5. Ayres, P. J.; Garrod, O.; Tait, S. A. S., and Tait, J. F.: Primary aldosteronism (Conn's syndrome), *in* Aldosterone, An International Symposium, ed. by A. F. Muller and C. M. O'Connor. Boston; Little, Brown, and Co., 1958, p.143.
6. Lieberman, A. H.; Slade, C. I.; Krück, F., and Luetscher, J. A., Jr.: Secretion of corticosteroids other than aldosterone in Conn's syndrome, *Clin. Res.* **6**: 93, 1958.
7. Venning, E. H.; Lucis, O. J.; Dyrenfurth, I., and Beck, J. C.: Effect of various pituitary and hypothalamic extracts on aldosterone secretion. Abstract No. 77, Program of the 40th Meeting of the Endocrine Society, San Francisco, 1958, p. 58.
8. Dorfman R. I.: Biosynthesis of steroids in hyperactive and tumour-bearing human glands, *Ciba Fndtn. Coll'q. Endocrinology* **12**: 62, 1958.
9. Lombardo, M. E.; Roitman, E., and Hudson, P. B.: Conversion of progesterone, 17-α-hydroxyprogesterone and 11-deoxycortisol to cortisol by the human adrenal gland *in vitro*, *J. Clin. Endocrinol. & Metab.* **16**: 1283, 1956.
10. Berliner, M. L.; Berliner, D. L., and Dougherty, T. F.: Metabolism of proges-

terone by adrenal tissue from patients with Cushing's syndrome and mammary carcinoma, *J. Clin. Endocrinol. & Metab.* **18:** 109, 1958.

1. Grant, J. K.; Symington, T., and Duguid, W. P.: Effect of adrenocorticotropic therapy on the *in vitro* 11β-hydroxylation of deoxycorticosterone by human adrenal homogenates, *J. Clin. Endocrinol. & Metab.* **17:** 933, 1957.
2. Mulrow, P. J.; Shmagranoff, G. L.; Lieberman, A. H.; Slade, C. I., and Luetscher J. A., Jr.: Stimulation of rat adrenocortical secretion by a factor present in human urine, *Endocrinology* **64:** 631, 1959.
3. Johnson, B. B.: Bioassay of adrenal cortical steroids on the basis of electrolyte excretion by rats: effects of 11-desoxy and 11-oxy-steroids, *Endocrinology* **54:** 196, 1954.
4. Drekter, I. J.; Pearson, S.; Bartczak, E., and McGavack, T. H.: A rapid method for the determination of total urinary 17-ketosteroids, *J. Clin. Endocrinol.* **7:** 795, 1947.
5. Sandberg, A. A.; Nelson, D. H.; Glenn, E. M.; Tyler, F. H., and Samuels, L. T.: 17-Hydroxycorticosteroids and 17-ketosteroids in urine of human subjects; clinical application of a method employing β-glucuronidase hydrolysis, *J. Clin. Endocrinol. & Metab.* **13:** 1445, 1953.
6. Axelrad, B. J.; Cates, J. E.; Johnson, B. B., and Luetscher, J. A., Jr.: Aldosterone in urine of normal man and of patients with oedema: increased recovery after hydrolysis with acid and with β-glucuronidase, *Brit. M. J.* **1:** 196, 1955.
7. Burton, R. B.; Zaffaroni, A., and Keutmann, E. H.: Paper chromatography of steroids. II. Corticosteroids and related compounds, *J. Biol. Chem.* **188:** 763, 1951.
8. Mader, I. J., and Iseri, L. T.: Spontaneous hypopotassemia, hypomagnesemia, alkalosis and tetany due to hypersecretion of corticosterone-like mineralocorticoid, *Am. J. Med.* **19:** 976, 1955.
9. Neher, R.: Aldosterone and other adrenocortical hormones in human adrenals and adrenal tumours, *in* Aldosterone, An International Symposium, ed. by A. F. Muller and C. M. O'Connor. Boston; Little, Brown, and Co., 1958, p. 11.
10. Goldsmith, R. S.; Bartter, F. C.; Rosch, P. J.; Meroney, W. H., and Herndon, E. G., Jr.: "Primary aldosteronism" associated with significant edema, *J. Clin. Endocrinol. & Metab.* **18:** 323, 1958.
11. Luetscher, J. A., Jr.: The syndrome of mineralocorticoid excess, *Ann. Int. Med.* **48:** 1424, 1958.
12. Mach, R. S.; Fabre, J.; Muller, A. F., and Neher, R.: Oedèmes par rétention de chlorure de sodium avec hyperaldostéronurie, *Schweiz. med. Wchnschr.* **85:** 1229, 1955.
13. Thorn, G. W.; Renold, A. E.; Froesch, E. R., and Crabbé, J.: Pathophysiology of edema, *Helvet. med. acta* **23:** 334, 1956.
14. Luetscher, J. A., Jr., and Lieberman, A. H.: Idiopathic edema with increased aldosterone output, *Tr. A. Am. Physicians* **70:** 158, 1957.
15. Roberts, S.: Corticosteroid-releasing activity in blood, *Ciba Fndtn. Colloq. Endocrinol.* **11:** 167, 1957.

SHORT METACARPAL OR METATARSAL BONES: PSEUDO-PSEUDOHYPOPARATHYROIDISM

H. HORTLING, M.D., E. PUUPPONEN, M.D. AND K. KOSKI, D.D.S.

Deaconess Hospital, II Medical Department, and Institute of Dentistry, University of Helsinki, Helsinki, Finland

ABSTRACT

The occurrence of short metacarpal or metatarsal bones is described in 1 patient who fulfilled the original description of pseudo-pseudohypoparathyroidism but without peripheral calcifications, in 1 patient with Turner's syndrome and in 6 persons of normal height distributed in two families. In none of these cases were signs of tetany observed. The frequency of short metacarpal bones in a general population consisting of 764 persons was found to be nil.

THE occurrence of short metacarpal or metatarsal bones was originally reported by Albright *et al.* (1) in 1942, in connection with the syndrome of pseudohypoparathyroidism (p.h.). Other typical symptoms of the syndrome were short stature, rounded face, soft-tissue calcifications and inability of the peripheral tissue to respond to the administration of the parathyroid hormone. In 1952 Albright *et al.* (2) reported a patient showing a normal response to parathyroid hormone but having all the other described characteristics. This syndrome was called pseudo-pseudohypoparathyroidism (p.p.h.). Others have also observed cases of p.p.h., but usually without soft-tissue calcifications (3–8). In 1959 Van der Werff ten Bosch (9) reported 8 further examples of the p.p.h. syndrome, 4 of which also involved gonadal dysgenesis. He suggested the name brachymetacarpal dwarfism (without tetany). Fromm (10) had already in 1956 criticized the name pseudo-pseudohypoparathyroidism. One may suppose that Albright introduced it in order to draw attention to the problem associated with it and did not intend it to be used forever.

This report concerns the occurrence of short metacarpal or metatarsal bones, or both: 1) in a patient fulfilling the original description of p.p.h., but without peripheral calcifications, 2) in one of 10 cases of Turner's syndrome, and 3) in 6 persons of normal height distributed in two families. There were no signs of tetany in any of these subjects. Out of a general population consisting of 764 persons the number having short metacarpal bones was found to be nil.

Received August 12, 1959.

CASE REPORTS

Case 1

This 16-year-old girl attended the medical department because of stunted growth. As far as the mother knew there were no similar abnormalities in the family. The patient was the only child in the family. Because of repeated tonsillitis, tonsillectomy was performed in 1952. In 1957 she was thought to suffer from hypothyrosis; her serum protein-bound iodine (PBI) level was then 2.9 μg. per 100 ml., and she had used thyroid since. Symptoms such as fatigue, headache and eczema of the hands had disappeared during thyroid therapy. The menarche occurred at 14 years and menstruation had been regular.

The patient (Fig. 1) was 144 cm. tall and her weight was 54 Kg. She had a rounded

FIG. 1. The patient in Case 1.

FIG. 2. Roentgenogram of hands in Case 1.

face and was thick-set. Mentally she was normal. In both hands the fourth finger was short; upon the formation of fists, there was dimpling over the fourth metacarpophalangeal joints. In roentgenograms the fourth metacarpal bone on both sides was very short (Fig. 2). The feet were normal. The bone age, according to Greulich and Pyle's tables, corresponded to the chronologic age. When thyroid therapy was discontinued for two months the serum PBI level was 4.1 μg. per 100 ml., cholesterol level 274 mg. per 100 ml., and basal metabolic rate −12 per cent. A normal estrogen effect was observed in the vaginal smear. A female chromosomal pattern was found in the granulocytes. The concentration of serum calcium was 9.7 and 10.0 mg. per 100 ml., and of inorganic phosphorus 3.5 and 4.1 mg. per 100 ml., respectively. No metastatic soft-tissue calcifications were detectable. There was no Chvostek sign and results of the Trousseau-hyperventilation test were negative. The Ellsworth-Howard test was performed but no significant increase was found in urinary phosphorus excretion. As the same negative effect was observed in a control subject when using the same batch of Parathormone Lilly, no significance can be attached to this result. An intravenous calcium infusion test was also

performed according to Howard (11). The excretion of calcium during the twenty-four hours preceding the administration of calcium was 81 mg., and during the twenty-four hours after the infusion it was 197 mg. The inorganic phosphorus values were 637 mg. and 616 mg., respectively. This result does not agree with the reported results in hypoparathyroidism.

Case 2

In a series of 10 cases of Turner's syndrome (gonadal dysgenesis), 1 patient showed a short left fourth metacarpal bone (Fig. 3). Roentgenograms of the feet were not available, as the patient was seen in 1951. This patient has been reported previously (12). She was 127 cm. tall and 24 years old at the time. She showed no clinical symptoms of tetany. The serum calcium level was 10.3 mg. per 100 ml., and inorganic phosphorus 2.6 mg. per 100 ml.

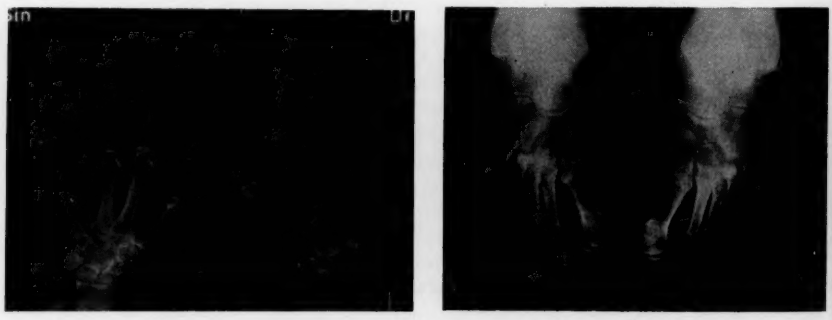
FIG. 3. Roentgenogram of hand in Case 2.

Case 3

Patients 3, 4 and 5 were from the same family. Patient 3 was a 71-year-old woman who was treated in the hospital because of cardiosclerosis. Quite by chance it was found that she had short fourth metacarpal and metatarsal bones on both sides (Fig. 4, a and b). Dimples were present over the fourth metacarpophalangeal joints (Albright's sign). This patient's height was 162 cm. Neither soft-tissue calcifications nor clinical signs of tetany could be found. Results of the Trousseau-hyperventilation test were negative. The serum calcium level was 9.2 mg. per 100 ml., and inorganic phosphorus 2.6 mg. per 100 ml.

Case 4

This woman was a sister of the patient in Case 3. She was 170 cm. tall, and had a short right fourth metatarsal bone (Fig. 5). She had no clinical symptoms of tetany. The serum calcium level was 9.2 mg. per 100 ml., and inorganic phosphorus 3.1 mg. per 100 ml. Results of the Trousseau-hyperventilation test were negative.



a b

FIG. 4. a) Roentgenogram of hands, and b) roentgenogram of feet, in Case 3.

Case 5

The patient was the daughter of the woman in Case 4. According to the mother, the daughter had the same abnormality in her hand as had the patient in Case 3. She was of normal height, and quite healthy.

FIG. 5. Roentgenogram of foot in Case 4.

Case 6

Patients 6, 7 and 8 were from the same family and were not related to the previous family. Patient 6 had short metacarpal and metatarsal bones. On both sides the hands showed typical dimples over the fourth metacarpophalangeal joints. She died in 1956 at the age of 74. She was the only child in the family, and was the mother of one of the authors (H.Hg). Her height was 168 cm. Unfortunately there were no pictures available to show the abnormality. During her life she had no clinical symptoms of tetany, and her serum calcium level was normal. During a hospital investigation which lasted several months, no soft-tissue calcifications could be observed, although many different roentgen studies were made.

Case 7

This 49-year-old male was 184 cm. tall. He was healthy and showed no clinical signs of tetany or soft-tissue calcifications. His fourth metatarsal bones, as may be seen in Figure 6, were very short. The serum calcium level was 10.0 mg. per 100 ml., and inorganic phosphorus 4.1 mg. per 100 ml. The Trousseau-hyperventilation test yielded negative findings.

Case 8

This healthy 41-year-old male was 178 cm. tall. He showed no clinical signs of tetany, the serum calcium level was 9.6 mg. per 100 ml., and results of the Trousseau-hyperventilation test were negative. His first right metatarsal bones were shorter than normal (Fig. 7). The four first toes on both feet appeared equally long.

FIG. 6. Roentgenogram of feet in Case 7.

FIG. 7. Roentgenogram of feet in Case 8.

The family tree of the family to which Patients 6–8 belonged is shown in Figure 8. None of the members of this family showed clinical signs of tetany. They all were of normal height and they did not have blue sclerae or other signs of osteogenesis imperfecta.

In a relatively normal population, two series of roentgenograms of the hand were checked in order to find the frequency of occurrence of anomalous metacarpal bones. The first series consisted of roentgenograms of the hands in 158 school children from 7 to 17 years of age, who were collected for another study in which the main purpose was to get samples for different age groups. The second series included 606 roentgenograms of the hands taken routinely in the orthodontic department of the Institute of

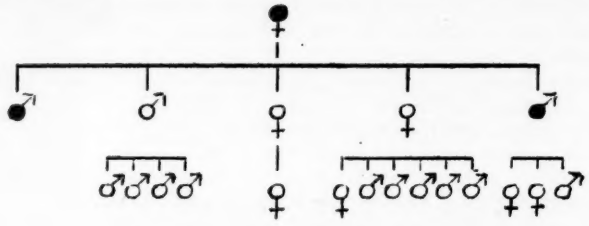


FIG. 8. The family tree of Cases 6–8.

Dentistry. The age range in this sample was from 5 to 33 years, the great majority of patients being from 8 to 16 years old. No anomalous metacarpal bones were found in these two series, which consisted of persons with no apparent endocrinal disturbances.

DISCUSSION

These observations add significantly to the knowledge of anomalous metacarpal or metatarsal bones, especially the fourth. It seems that the anomaly is more likely to occur without rather than with disturbances of calcium metabolism, since no cases of tetany were observed in this group of persons. Albright's observation of 1 case that he named pseudo-pseudohypoparathyroidism is of interest in this respect.

According to previous reports, short metacarpal or metatarsal bones are as a rule combined with stunted growth. Van der Werff ten Bosch thus coined the term brachymetacarpal dwarfism. In our series of 8 persons with short metacarpal or metatarsal bones only 2 showed stunted growth whereas the 6 others were of normal height.

In checking the frequency of abnormal metacarpal bones in 764 roentgenograms of the hands of apparently normal persons, we found it to be nil. This finding allows the forming of a definite opinion with regard to the general significance of short metacarpal or metatarsal bones, even if the meta-

tarsal bones were not included in the series of 764 persons. The conclusion, backed by the reported observations, is as follows:

The anomaly is quite rare. It is often hereditary, as has already been pointed out by Seringe and Tomkiewicz (7). It may be associated with various disorders also known to be hereditary, such as pseudohypoparathyroidism and a moderate form of stunted growth. Other disturbances associated with this hand or foot anomaly are Turner's syndrome and osteogenesis imperfecta (7). A thick-set body build and rounded face seem also to be common, and probably a number of other characteristics may be found. In the other members of the family to which Cases 6–8 belonged, we often observed crooked little fingers and slight anomalies in the hand or foot bones. Accessory metatarsal bones, as in Case 8 (Fig. 7), were found in at least 2 other members of the same family.

Short metacarpal or metatarsal bones thus seem to have no special clinical significance. Their occurrence may, however, indicate that it would be worth while to look for other congenital disorders, including those of the endocrine system.

Addendum

After this manuscript was submitted for publication, a paper by R. Archibald, N. Finby and F. De Vito (Endocrine significance of short metacarpals, *J. Clin. Endocrinol. & Metab.* **19:** 1312–1322 (Oct.) 1959) came to our attention. These authors studied the frequency of the metacarpal sign in 2,594 patients who attended the outpatient department of the child growth-study and endocrine clinics of the Rockefeller Hospital during the past ten years. The sign is present when a line drawn tangentially to the circumference of the heads (distal ends) of the fourth and fifth metacarpals runs through the distal end of the third metacarpal, whereas as a rule it passes distal to the third metacarpal. They noted frequent occurrence of the sign in patients with gonadal dysgenesis (in 82 per cent of 17 cases); moreover, in males (Klinefelter's syndrome excluded) it occurred much more frequently in association with some gonadal anomaly than when the gonads were normal. The sign was observed in 105 males, and 136 others exhibited a borderline condition. Though an occasional patient among these was taller than average, the mean height was appreciably less than the average expected height based on the skeletal age.

REFERENCES

1. Albright, F.; Burnett, C. H.; Smith, P. H., and Parson, W.: Pseudo-hypoparathyroidism—An example of "Seabright-bantam syndrome." Report of three cases, *Endocrinology* **30:** 922, 1942.
2. Albright, F.; Forbes, A. P., and Henneman, P. H.: Pseudo-pseudohypoparathyroidism, *Tr. A. Am. Physicians* **65:** 337, 1952.
3. Miles, J., and Elrick, H.: Pseudo-pseudohypoparathyroidism: report of a new case, *J. Clin. Endocrinol. & Metab.* **15:** 576, 1955.
4. Roche, M.: A case of pseudo-pseudohypoparathyroidism, *J. Clin. Endocrinol. & Metab.* **15:** 964, 1955.
5. Wallach, S.; Englert, E., and Brown, H.: The syndrome of pseudo-pseudohypoparathyroidism, *A.M.A. Arch. Int. Med.* **98:** 517, 1956.

6. McNeely, W. F.; Raisz, L. G., and LeMay, M.: Dyschondroplasia with soft tissue calcification and ossification, and normal parathyroid function ("pseudo-pseudohypoparathyroidism"), *Am. J. Med.* **21**: 649, 1956.
7. Seringe, P., and Tomkiewicz, S.: La dystrophie d'Albright: pseudo-pseudo-hypoparathyroïdisme de cet auteur, *Sem. hôp. Paris* **33**: 1092, 1957.
8. Smulyan, H., and Raisz, L. G.: Pseudo-pseudohypoparathyroidism with unusual features, *J. Clin. Endocrinol. & Metab.* **19**: 478, 1959.
9. Van der Werff ten Bosch, J. J.: The syndrome of brachymetacarpal dwarfism ("pseudo-pseudohypoparathyroidism"), *Lancet* **1**: 69, 1959.
10. Fromm, G. A.: Concerning the term "pseudo-pseudohypoparathyroidism," *J. Clin. Endocrinol. & Metab.* **16**: 293, 1956.
11. Howard, J. E.; Hopkins, T. R., and Connor, T. B.: The use of intravenous calcium as a measure of activity of the parathyroid glands, *Tr. A. Am. Physicians* **65**: 351, 1952.
12. Hortling, H., and Jäämeri, K. E. U.: Therapeutic trials in dwarfs of the type known as Turner's syndrome, *Acta endocrinol.* **13**: 97, 1953.

ACUTE DIABETIC EPISODE WITH REMISSION: REPORT OF A CASE*

RICHARD W. BARR, LT. (MC) USNR†
U. S. Naval Hospital, Quantico, Virginia

ABSTRACT

An unusual case is reported of a 39-year-old U. S. Marine in whom a remission occurred following an acute episode of diabetic acidosis and coma. The patient responded well to therapy for coma, and was treated with insulin for several weeks. Gradually, hypoglycemia developed with insulin therapy. The dosage of insulin was therefore gradually diminished and at present he requires none. His oral five-hour glucose tolerance is normal. However, a five-hour glucose tolerance test following administration of cortisone reveals latent diabetes.

REPORTS of remission of diabetes mellitus with previous episodes of coma and acidosis are rare. A few such cases have appeared in the literature recently (1, 2). Harwood's interesting article includes a thorough review of the literature, with a plea for clinicians to aim for remission in their treatment of early diabetes (1). The following case is unusual in that it concerns an acute diabetic episode followed by remission. At the present time the remission has lasted for twenty-four months; the patient is in good health and has a normal blood sugar level without the use of insulin.

CASE REPORT

D.S., a 39-year-old T/Sgt., U.S.M.C. with sixteen years and nine months of service was referred on May 20, 1957, to the U. S. Naval Hospital, Quantico, Virginia from the Marine Corps Schools Dispensary with the tentative diagnosis of hyperventilation. During the previous three months he had noticed polydipsia, polyphagia and a 24-pound weight loss (155 to 131 pounds). He had also experienced excessive blurring of vision and difficulty in focusing. The past history was unremarkable. One sister died during childbirth and was discovered to be diabetic during that pregnancy.

The patient was a well developed, thin white male who was semicomatose and had obvious Kussmaul-type breathing. His pulse was 136 per minute, and his height was 68 inches. There was a strong odor of acetone from his breath. The skin and mucous membranes were noticeably dry. The eye balls were soft. There was no evidence of an infectious process, abdominal spasm or tenderness. The remainder of the findings on physical examination were within normal limits.

Received August 29, 1959.

* The opinions expressed herein are the private ones of the author and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

† *Present address:* U. S. Naval Hospital, Chelsea 50, Massachusetts.

Urinalysis revealed 4-plus sugar and 4-plus acetone. Albuminuria (100 mg.) was present, and the sediment contained a few granular casts. The concentration of blood sugar was over 550 mg. per 100 ml. (Folin-Wu), and the serum carbon dioxide content was 4 mEq. per liter. The diagnosis was changed to diabetes mellitus.

During the next twelve hours he received a total of 195 units of regular insulin and 4 liters of fluids parenterally (mostly 0.85 per cent solution of sodium chloride). Insulin was administered every two hours in a dosage adjusted according to the degree of glycosuria. After six hours of treatment the blood sugar level fell to 300 mg. per 100 ml. At the end of twelve hours only a trace of sugar remained in the urine, and there was no acetonuria. He was given a 1500-calorie soft diet (150–70–70). After the sixth hospital day, the fasting blood sugar level remained normal under treatment with 30 U. of NPH insulin daily. On the second hospital day an ophthalmic consultant discovered severe hyperopia with 20/200 vision, due to marked dehydration of the lenses. Manifest refraction revealed O.D. +3.5 and O.S. +4.0. There was no diplopia. The patient was given a pair of Pickening multiple pin-hole spectacles with good visual results.

By the end of the second hospital week he was noted to have persistently low fasting blood sugar values (60 to 66 mg. per 100 ml.). The dosage of NPH insulin was progressively decreased and finally discontinued on June 18, 1957.

For the next three weeks as an out-patient, no glycosuria or acetonuria was detected with routine examinations four times daily. Careful observation of the patient with questioning failed to reveal any surreptitious use of insulin. He maintained a normal concentration of fasting blood sugar during this time, with a 2500-calorie diet (American Diabetes Association) and no insulin.

On July 11, a five-hour oral glucose tolerance test showed: fasting blood sugar level, 79 mg. per 100 ml.; half hour, 185 mg.; two hours, 119 mg.; three hours, 64 mg.; four hours, 70 mg.; and five hours, 86 mg. Respective urine specimens were free of sugar. Skull and chest roentgenograms were normal. The basal metabolic rate was within normal limits. The 24-hour excretion of 17-ketosteroids, the level of serum amylase, and the amount of fat in the stool were reported as normal. A flat x-ray plate of the abdomen failed to reveal any pancreatic calcification. On August 1, 1957 (six weeks after the omission of insulin), another five-hour glucose tolerance test was performed. Two days previously and on the day of the test the patient was given 50 mg. of cortisone orally four times daily. Resulting values for blood sugar were: fasting, 72 mg. per 100 ml.; half hour, 156 mg.; one hour, 218 mg.; two hours, 213 mg.; three hours, 213 mg.; four hours, 165 mg.; and five hours, 115 mg. At no time was sugar noted in the urine. Liver function tests yielded normal results. A three-hour oral glucose tolerance test performed on the patient's brother showed a normal blood sugar curve.

The patient was then assigned to limited duty for a period of one year. The fasting blood sugar level was determined monthly, and urinalysis for sugar was performed four times daily. Body weight was 133 pounds upon discharge. On September 14, 1958, the patient was admitted for re-evaluation. In the fourteen-month interval he had done well in all respects. He had followed his diet faithfully, and had examined his urine four times daily with consistently negative results. Fasting blood sugar levels at monthly intervals ranged from 80 mg. to 130 mg. per 100 ml., with an average of 110 mg. per 100 ml. His weight had remained stable, and appetite had been good. He denied any visual disturbances or intercurrent infections. A complete blood count and results of urinalysis were within normal limits. A three-hour oral glucose tolerance test showed the following blood sugar levels: fasting, 95 mg. per 100 ml.; half hour, 167 mg.; one hour, 215 mg.; two hour, 125 mg.; and three hours, 95 mg. The respective urines contained no sugar.

The level of blood urea nitrogen was 10.5 mg. per 100 ml. Intravenous pyelograms, Addis counts of the urinary sediment and Fishberg concentration tests all yielded negative results. An electrocardiogram and chest roentgenogram were normal. The ophthalmic consultant found 20/15 vision in both eyes.

The patient was well indoctrinated in the principles of diabetic management, extremely conscientious, and well motivated for military service, hoping to complete at least nineteen years and six months of active duty. At the present time (twenty-four months after his admission in a state of acidosis) he continues to examine his urine daily for sugar and to undergo monthly fasting blood sugar determinations, which continue to be within normal limits. He remains on active limited duty.

DISCUSSION

Recovery from diabetes mellitus following an episode of coma and acidosis is rare. This is reasonable, in view of the fact that a severe diabetic condition such as coma indicates that the beta cells of the pancreatic islets are either severely damaged or exhausted. This case is especially interesting since the patient was not obese nor partaking of a heavy carbohydrate diet prior to his initial hospital admission. There was a family history of diabetes, but the cause for his diabetic acidosis is unknown. Although the serum amylase level was not determined at the time of the initial admission, no other criteria (aside from dehydration) of coma with acute pancreatitis as listed by Tully and Lowenthal (3) were present. According to Root (4), sweating is a common accompaniment of coma associated with pancreatitis.

This patient is an extremely well organized, alert person who is very conscientious about pleasing and helping others. Perhaps one could hypothesize that some phase of stress of military life caused development of the overt state of diabetes. The existence of a latent diabetic state seems apparent on the basis of the failure of his pancreatic islets to meet the demand for insulin production when carbohydrate metabolism was influenced by administration of cortisone in moderately large dosage during the five-hour glucose tolerance test. Although the patient has remained on a program of limited duty, the signs and symptoms of diabetes have not appeared. Actually, the original purpose in treating this patient was not to aim for remission. Neither I nor the other doctors who attended him can claim credit for the remission, nor can we explain his recovery. Undoubtedly, the relatively short duration of symptoms along with the patient's meticulous cooperation contributed to his present satisfactory state. Apparently this is the sixth reported case in an adult of remission of diabetes following acidosis (1, 2, 5, 6)

REFERENCES

1. HARWOOD, R.: Severe diabetes with remission: report of case and review of literature, *New England J. Med.* **275**: 257 (Aug. 8) 1957.

2. JOHNSON, W. M.: Acute diabetic episode with remission: report of two cases, *New England J. Med.* **258:** 234 (Jan. 30) 1958.
3. TULLY, G. T., and LOWENTHAL, J. J.: Diabetic coma of acute pancreatitis, *Ann. Int. Med.* **48:** 310 (Feb.) 1958.
4. ROOT, H. F.: Diabetic coma and acute pancreatitis with fatty livers, *J.A.M.A.* **108:** 777 (March 6) 1957.
5. CHENG, T. O.; JAHRAUS, R. C., and TRAUT, E. F.: Extreme hyperglycemia and severe ketosis with spontaneous remission of diabetes mellitus, *J.A.M.A.* **152:** 1531 (Aug. 15) 1953.
6. DEL GRECO, F., and SCAPELLATO, L.: Transient diabetes with coma following short term excessive consumption of carbohydrate: case report, *Diabetes* **2:** 457 (Sept.–Oct.) 1953.

Letters to the Editor

LEVELS OF SERUM PROTEIN-BOUND IODINE IN PATIENTS WITH METASTATIC CARCINOMA OF THE BREAST

To the Editor:

During a study of the endocrinologic effects of various steroid hormones administered to women with progressive metastatic carcinoma of the breast, it was noted that pretreatment levels of serum protein-bound iodine (PBI) were elevated above normal. A study was undertaken to determine the significance of this observation.

MATERIALS AND METHODS

Three groups of postmenopausal women were studied (Table 1). Group A consisted of 46 women with progressive metastatic mammary carcinoma; Group B, of 27 women who had undergone surgery of the breast for carcinoma at least three years prior to the study and were apparently free of carcinoma; and Group C, of 20 healthy women. In Group A, 30 women had undergone radical mastectomy and 16 had primary inoperable disease. The predominant metastatic lesion was visceral in 17, osseous in 16 and local in 13. The mean ages and the mean number of years after the menopause were comparable in the three groups. None of the patients had recently received any medications containing iodine or thyroid hormone, nor had they had any recent radiographic examinations requiring the administration of organic iodides. The levels of serum PBI were measured by a modification of the method of Zak *et al.* (1, 2).

RESULTS

The levels of serum protein-bound iodine in the various groups are shown in Table 1. In Group A, the levels ranged from 4.3 to 10.4 μg. per 100 ml. with a mean value of 7.4 ± 1.3 μg. per 100 ml., compared to a mean level of 6.3 ± 1.0 μg. per 100 ml. in control Group B, and a mean level of 6.5 ± 1.2 μg. per 100 ml. in Group C. The difference between the means for Groups A and B was highly significant, $P < 0.001$, as was the difference between the means for Groups A and C, $P < 0.01$. There was no statistically significant difference between the mean values for Groups B and C, $P > 0.95$.

* This work was supported in part by Grants CY3601 and CY3358 from The National Cancer Institute, The National Institutes of Health, United States Public Health Service.

TABLE 1. LEVELS OF SERUM PROTEIN-BOUND IODINE IN HEALTHY POSTMENOPAUSAL WOMEN AND POSTMENOPAUSAL WOMEN WITH CARCINOMA OF THE BREAST*

	Progressive metastatic carcinoma of breast (Group A)	"Cured" carcinoma of breast (Group B)	Healthy (Group C)
Number of patients	46	27	20
Age (yrs.)	60 (32–81)†	66 (38–79)	56 (36–73)
Years postmenopausal	10.7 (0.2–40)	12.9 (2–29)	9.5 (1–24)
Years after surgery	4.5 (0.2–27)	10.1 (3–29)	
Serum PBI (μg./100 ml.)	7.4± 1.3 (4.3–10.4)‡	6.3±1.0 (4.5–8.2)	6.5±1.2 (4.6–9.4)
Standard Error	0.19	0.20	0.26

* P values for Groups A and B, <0.001; for Groups A and C, <0.01; for Groups B and C, >0.95. P values determined by Student's *t* test.

† Mean and range.

‡ Mean ± standard deviation and range.

There was no correlation between the serum PBI level and the locus of the dominant metastatic lesions in Group A. Moreover the PBI levels in 7 patients who survived less than one month were the same as in 7 patients who survived an average of seventeen months.

Serum thyroxine-binding protein (TBP) was measured[1] in 3 patients in Group A whose PBI levels were 5.9, 7.2 and 8.3 μg. per 100 ml. The TBP capacities were 0.33, 0.37 and 0.61 μg. of thyroxine per ml. respectively compared to a control TBP capacity of 0.26 μg. of thyroxine per ml. (3).

DISCUSSION

In the group of patients with advanced metastatic carcinoma of the breast, the mean levels of serum protein-bound iodine were significantly higher than those in a control group of patients "cured" of mammary cancer or in healthy women of similar age and menopausal status. The PBI

[1] Studies performed through the courtesy of Dr. Jacob Robbins, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, United States Public Health Service.

values for the latter two groups were indistinguishable from each other and from the previously established normal mean value for serum PBI determined in our laboratory. Preliminary studies indicate that serum TBP capacity may be elevated also in the subjects with advanced metastatic breast cancer. These studies are being carried out in additional patients.

Other investigators have determined serum PBI levels in acutely and chronically critically ill patients and noted values lower than the normal mean. The lowest values were obtained in the chronically ill patients (4). Other types of sick patients have shown abnormalities in serum thyroxine-binding pre-albumin and in the cellular uptake of labeled thyroxine and triiodothyronine, similar to those observed in thyrotoxic patients (5, 6). The uptake by red blood cells of labeled triiodothyronine is increased in thyrotoxicosis and in euthyroid patients with extensive metastatic malignant lesions (7).

Elevation of the levels of serum PBI in conditions other than hyperthyroidism occurs in pregnancy (8) and in patients receiving estrogens (9). In these situations, a rise in TBP also occurs (10, 11) but the uptake of labeled triiodothyronine by red blood cells is diminished (7).

Statistically, the mean levels of the serum protein-bound iodine are elevated in patients with progressive metastatic mammary carcinoma. No conclusions can be drawn at present as to the significance of this change or the mechanism by which the alteration in circulating thyroid hormone takes place.

ANNE C. CARTER, M.D.
ELAINE BOSSAK FELDMAN, M.D.
with the technical assistance of
HAROLD L. SCHWARTZ, B.S.

Department of Medicine,
State University of New York,
College of Medicine at New York City,
Medical Service (Division II),
Kings County Hospital,
Brooklyn, N.Y.,
September 28, 1959

REFERENCES

1. ZAK, B.; WILLARD, H. H.; MEYERS, G. B., and BOYLE, A. J.: Chloric acid method for determination of protein-bound iodine, *Analyt. Chem.* **24**: 1345, 1952.
2. BODANSKY, O.; BENUA, R. S., and PENNACHIA, G.: A rapid procedure for determination of total and protein-bound iodine in serum, *J. Lab. & Clin. Med.* **30**: 375, 1958.
3. ROBBINS, J.: Reverse-flow zone electrophoresis. A method for determining the thyroxine-binding capacity of serum protein, *Arch. Biochem & Biophys.* **63**: 461, 1956.
4. ENGSTROM, W. W., and MARKARDT, B.: The effects of serious illness and surgical

stress on the circulating thyroid hormone, *J. Clin. Endocrinol. & Metab.* **15:** 953, 1955.

5. Richards, J. H.; Dowling, J. T., and Ingbar, S. H.: Alterations in plasma transport of thyroxine in sick patients and their relation to the abnormality in Graves' disease, (abstract). *J. Clin. Invest.* **38:** 1035, 1959.
6. Ingbar, S. H., and Freinkel, N.: The thyroxine-binding proteins of plasma and their role in the regulation of thyroxine metabolism, *Recent Progr. Hormone Res.* **16:** (in press) 1960.
7. Hamolsky, M. W.; Golodetz, A., and Freedberg, A. S.: The plasma protein-thyroid hormone complex in man. III. Further studies on the use of the *in vitro* red blood cell uptake of I^{131}-*l*-triiodothyronine as a diagnostic test of thyroid function, *J. Clin. Endocrinol. & Metab.* **19:** 103, 1959.
8. Heinemann, M.; Johnson, C. E., and Man, E. B.: Serum precipitable iodine concentrations during pregnancy, *J. Clin. Invest.* **27:** 91, 1948.
9. Engstrom, W. W.; Markardt, B., and Liebman, A.: Effect of estrogens on serum precipitable iodine, *Proc. Soc. Exper. Biol. & Med.* **81:** 582, 1952.
10. Dowling, J. T.; Freinkel, N., and Ingbar, S. H.: Effect of diethylstilbestrol on the binding of thyroxine in serum, *J. Clin. Endocrinol. & Metab.* **16:** 1491, 1956.
11. Robbins, J., and Nelson, J. H.: Thyroxine-binding by serum protein in pregnancy and in the newborn, *J. Clin. Invest.* **37:** 153, 1958.

POSSIBLE ERRORS IN THE DETERMINATION OF RED BLOOD CELL UPTAKE OF I^{131}-TRIIODOTHYRONINE

To the Editor:

The *in vitro* red blood cell uptake of I^{131}-*l*-triiodothyronine (I^{131}-Trit) was introduced as a diagnostic test of thyroid function by Hamolsky *et al.* (1, 2). In this procedure, I^{131}-Trit is mixed with whole blood and incubated at 37° C. for two hours. One-milliliter aliquots of whole blood are then removed and the radioactivity of each aliquot is determined before and after 5 washes with 10 ml. of isotonic saline. The percentage of whole blood radioactivity which remains with the washed red blood cells is calculated and corrected to a hematocrit of 100. The procedure appears to have some advantages over the usual I^{131} thyroid function tests. However, results beyond the range of normal were not uncommonly found when euthyroid patients were studied in this laboratory and values for a given patient could not always be duplicated on successive weeks. Further evaluation of the various steps of the procedure revealed some possible sources of error, as demonstrated in the following studies.

To fresh heparinized whole blood in an Erlenmeyer flask, I^{131}-Trit was added to yield a concentration of 15×10^{-4} μg. per ml. and the mixture was stoppered and incubated at 37° C. for two hours with frequent mixing. Following the incubation, 1-ml. aliquots of whole blood were transferred to

test tubes for study. A sample was also taken for hematocrit determination. The remaining blood was centrifuged and duplicate 1-ml. samples of plasma were transferred to test tubes for counting and indirect calculation of the erythrocyte uptake of I^{131}-Trit.

Following the initial count of radioactivity, the whole blood samples were washed repeatedly with 10 ml. of isotonic sodium chloride solution and the activity remaining after 2, 4, 5, 6, 8 and 10 washings was determined. In Table 1 the average values of duplicate determinations on blood from 20 euthyroid patients is shown. The results are listed both as the percentage of whole blood activity remaining bound to erythrocytes and the same result corrected to a hematocrit of 100. The latter value is used in the procedure proposed by Hamolsky *et al.* to correct for variations in hematocrit, and both values will be shown in tables throughout this study. Table 1 shows that the activity remaining with the erythrocytes decreases with each washing. The data plotted on semi-logarithmic paper yielded a straight line which was extrapolated to zero to obtain the zero wash value shown as a predicted value.

As listed in the last column of Table 1, the I^{131}-Trit activity incorporated with the erythrocytes after 5 washes was 43 per cent of the predicted activity before washing. The I^{131}-Trit uptake of the red blood cells before washing was also calculated indirectly from the activity present in the plasma at the end of incubation, using the following formula:

$$\frac{\text{Whole blood} - \left[\text{plasma} \times \left(\frac{100 - \text{hematocrit}}{100}\right)\right]}{\text{Whole blood}} = \text{RBC uptake}$$

For the same 20 patients listed in Table 1, the average red blood cell uptake calculated in this way was 15.3 per cent. This compares to the extrapolated value of 14 per cent shown in Table 1, and the values would be closer if the hematocrit were corrected for the plasma content of the packed red cells. These results, if corrected to 100 hematocrit, would be 33.5 and 30.4 per cent, and are greater than twice the normal value obtained by Hamolsky after 5 washes, *viz*, 11–17 per cent, average 13.9 per cent. The average of 13.1 per cent after 5 washes in this study is quite comparable. It is therefore evident that the amount of I^{131}-Trit remaining with the erythrocytes after 5 washes does not represent an end-point but is an arbitrary value on a first-order reaction curve. The amount of I^{131}-Trit removed from the red cells by any one washing is proportional to the amount present at the beginning of the wash. Excessive washing will shift a low-normal value into the hypothyroid range and inadequate washing will shift a high-normal value into the hyperthyroid range. Hyperthyroid and hypothyroid values could be similiarly affected.

TABLE 1. I^{131}-*l*-TRIIODOTHYRONINE ACTIVITY REMAINING IN ERYTHROCYTES AFTER MULTIPLE SALINE WASHES OF THE BLOOD FROM 20 EUTHYROID PATIENTS

No. of washes	Erythrocyte I^{131}-Trit activity		
	%	%/100 hematocrit	% of predicted
0* (predicted)	14.0	30.4	—
2	10.1	22.1	72.7
4	6.8	14.8	48.7
5	6.0	13.1	43.1
6	5.3	11.5	37.8
8	3.9	8.6	28.3
10	2.7	5.9	19.4

* See text.

The effect of other variables was studied. The volume of saline wash was varied. Five washes of 10 ml. or 5 ml. were carried out on paired duplicates. The average result obtained on the blood of 6 patients is shown in Table 2. One patient had untreated myxedema and this accounts for the low average values. Using the 5-ml. wash, the result in this patient was 12.1 per cent; using the 10 ml. wash, the result was 9.8 per cent. In all cases studied, the difference between results with the 10-ml. and the 5-ml. wash was relatively constant.

To determine the importance of the time taken to perform the washes, an exaggerated study was carried out: One group of samples was washed routinely. A second group was washed in the same way for two washes then isotonic sodium chloride was added, mixed, and the mixture allowed to stand for one hour before the third wash. Saline was again added, and the mixture allowed to stand (with mixing) for two hours before centrifuging and counting as the fourth wash. The results in Table 3 show an increased loss when the tubes are allowed to stand between washings. It appears to be important that the washing be continued to completion without delaying interruptions.

TABLE 2. I^{131}-*l*-TRIIODOTHYRONINE ACTIVITY REMAINING IN ERYTHROCYTES AS AFFECTED BY VOLUME OF WASH

Volume of saline wash	Erythrocyte I^{131}-Trit activity after 5 washes	
	%	%/100 hematocrit
5 ml.	6.86	13.8
10 ml.	5.62	11.6

TABLE 3. I^{131}-*l*-TRIIODOTHYRONINE ACTIVITY REMAINING IN ERYTHROCYTES AFTER RAPID *vs.* DELAYED WASHING

Method of washing	Erythrocyte I^{131}-Trit activity (%) after			
	2 washes*	3 washes	4 washes	5 washes
Rapid	9.93			6.3
Delayed	10.05	7.42	5.60	

* Procedure was the same through the first and second washes.

A comparison of washing with saline at 25° C. and 2° C. is shown in Table 4. The percentage activity remaining after 2 and 5 washes of 10 ml. each is shown for 8 paired duplicates. More I^{131}-Trit activity remained bound to the red cells at 2° C. and the average value approached the upper limits of normal. Two of the 8 values were in the hyperthyroid range after 5 washes at 2° C. Although the effect of washing with saline at a temperature higher than 25° C. was not studied, a more rapid removal of I^{131}-Trit could be predicted. For consistent results it would seem advisable to maintain a fixed temperature for the washes.

To determine that the decrease in red cell I^{131}-Trit activity after washing did not represent actual loss of cells, the hematocrit was determined of the cells remaining after 10 washes and compared to the hematocrit of the original whole blood. This was done for 16 determinations. The volume of packed cells from 1 ml. of washed whole blood was multiplied by 0.7, which is the volume of the hematocrit tube. The average hematocrit after 10 washes was found to be 43.5 per cent compared to an average value of 46.0 per cent for the original whole blood. The loss, if any, was slight.

In summary, the amount of I^{131}-triiodothyronine which remains with the erythrocytes after 5 saline washes appears to be an arbitrary value representing less than half of the amount incorporated with the red cells at the end of incubation. Each successive wash removes about 15 per cent of the

TABLE 4. EFFECT OF WASH TEMPERATURE ON ERYTHROCYTE I^{131}-*l*-TRIIODOTHYRONINE ACTIVITY AFTER 2 AND 5 WASHES

Temperature of saline	Erythrocyte I^{131}-Trit activity after			
	2 washes		5 washes	
	%	%/100 hematocrit	%	%/100 hematocrit
25° C.	9.9	21.5	6.3	13.3
2° C.	11.0	24.0	7.6	16.5

activity which remains after the preceding wash. If the time between washes, the temperature of the saline wash or the volume of saline is varied, the result is altered. Any one, or a combination of these variables may shift a borderline value into a higher or lower category. In using such a procedure without a definite end-point it seems that fairly exact conditions of washing must be followed in order to obtain reproducible results. Lack of knowledge of these variables may account for some abnormal results. The entire difficulty would be overcome if the triiodothyronine were fixed to the erythrocytes and could not be washed away. A study is in progress to determine if this can be done.

ROBERT C. MEADE, M.D.

Veterans Administration Center,
Radioisotope Service,
Wood, Wisconsin,
August 5, 1959

REFERENCES

1. HAMOLSKY, M. W.; STEIN, M., and FREEDBERG, A. S.: The thyroid hormone-plasma protein complex in man. II. A new *in vitro* method for study of "uptake" of labelled hormonal components by human erythrocytes, *J. Clin. Endocrinol. & Metab.* **17**: 33, 1957.
2. HAMOLSKY, M. W.; GOLODETZ, A., and FREEDBERG, A. S.: The plasma protein-thyroid hormone complex in man. III. Further studies on the use of the *in vitro* red blood cell uptake of I^{131}-*l*-triiodothyronine as a diagnostic test of thyroid function, *J. Clin. Endocrinol. & Metab.* **19**: 103, 1959.

The Endocrine Society

The 1960 Annual Meeting

The Forty-Second Annual Meeting of The Endocrine Society will be held in the Eden Roc Hotel, Miami Beach, Florida, Thursday, Friday and Saturday, *June 9, 10 and 11, 1960.*

The Chairman of Local Arrangements is Dr. Kenneth Savard.

Scientific Sessions will be held from 9:00 A.M. to 5:00 P.M. daily and in addition there will be simultaneous afternoon sessions. The annual dinner is scheduled for Friday, June 10, at 7:30 P.M. preceded by cocktails at 6:30 P.M.

All members are urged to make their hotel reservations immediately. The Eden Roc will hold 250 bedrooms and the adjoining hotels, the Montmartre Hotel and Lucerne Hotel, will hold 75 rooms each for members until May 1, 1960, after which time the hotel will not guarantee further reservations. Therefore it is imperative that you make your reservations early, directly with the hotel, advising them of time and date of arrival and departure. If you plan on remaining through June 12 for the American Medical Association and other meetings, it is imperative that your reservation be made from June 12 on, directly through the A.M.A. Housing Bureau, c/o Miami Beach Convention Bureau, 1700 Washington Avenue, Miami Beach, Florida, since no reservation beyond June 12 can be accepted directly by the hotels. Make your reservations early and avoid disappointment. Hotel reservation card will be mailed to members shortly after January 1, 1960.

Final program, membership card and advance registration forms will be sent on May 1, 1960 to members whose current dues have been paid.

The 1960 Awards

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

Fred Conrad Koch Award

In 1957 a substantial legacy was bequeathed to the Society by the late Elizabeth Koch for the purpose of establishing the Fred Conrad Koch Memorial Fund in memory of her late husband, Distinguished Service Professor of Physiological Chemistry at the University of Chicago and pioneer in the isolation of the androgens. This is the highest honor of The Endocrine Society and is represented by a medal known as the Koch Medal of The Endocrine Society. The medal, as well as an honorarium of $3,500, is to be given annually to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

This Award was first presented in 1959, to Dr. W. W. Swingle. The Koch Medal has replaced the Medal of The Endocrine Society which was established in 1954 and presented to Dr. Carl R. Moore in 1955, Dr. Frederick L. Hisaw in 1956, Dr. Joseph C. Aub in 1957 and Dr. I. L. Chaikoff in 1958. The Medal of the Endocrine Society replaced the E. R. Squibb Award which was formerly the highest honor bestowed by the Society. Past recipients of the Squibb Award were Dr. George W. Corner in 1940, Dr. Philip E. Smith in 1941, Dr. Fred C. Koch in 1942, (no award in 1943), Dr. E. A. Doisy in 1944, Dr. E. C. Kendall in 1945, Dr. Carl G. Hartman in 1946, Drs. Carl F. and Gerty T. Cori in 1947, Dr. Fuller Albright in 1948, Dr. Herbert M. Evans in 1949, Dr. C. N. H. Long in 1950, Dr. J. B. Collip in 1951, Dr. James H. Means in 1952 and Dr. David Marine in 1953.

The Ciba Award

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross; 1956—Dr. Alfred M. Bongiovanni; 1957—Dr. Nicholas S. Halmi; 1958—Dr. Monte A. Greer; 1959—Dr. Gordon L. Farrell. Prior to 1952 the Award was $1,200. It has now been increased to $2,500.

The Ayerst and the Squibb Fellowships

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional

promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

The Upjohn Scholar of the Endocrine Society

The Council of The Endocrine Society has established a category of Scholars. A grant has been made available through the generosity of the Upjohn Company, and will be awarded to an established investigator or teacher in the field of endocrinology who wishes to extend the opportunities for work either in this country or abroad.

The award will not exceed $2,500 annually and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel. maintenance and other expenses.

Nominations

Nominations for the Fred Conrad Koch Award, the Ciba Award, and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *October 15* each year.

Proposals for appointments as a Scholar of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *October 15* each year.

First International Congress of Endocrinology

The First International Congress of Endocrinology will be held in *Copenhagen, Denmark, July 18–23, 1960.*

United States members of the International Executive Committee are Dr. Warren O. Nelson and Dr. Leo T. Samuels, Dr. Gregory Pincus is Chairman of the Program Committee. The tentative program consists of ten symposia covering the general field of endocrinology plus round-table discussions and a number of submitted papers. Those desiring to submit papers should immediately contact Dr. Gregory Pincus, Worcester Foundation, Shrewsbury, Massachusetts. There will be a number of interesting scientific and technical exhibits.

In order to facilitate travel arrangements to the Copenhagen Convention, the Council of The Endocrine Society at its San Francisco meeting designated THOMAS COOK & SON as our authorized travel agents. They will in turn offer especially designed All Expense Paid Tours to Europe, including hotel reservations for the convention, as well as offering independent itineraries for any of our members who desire to travel separately by air or by steamship.

Registration is open to anyone interested in endocrinology at an approximate fee of $28 U.S. Members of the families of those attending may register as associates at an approximate fee of $14 U.S., fee to be paid at the time of registration or in advance to the American Express Company, which has been designated by the Executive Committee of the International Congress as official financial and travel agent. There will be a number of social functions and other entertainment provided for all registrants.

As you all know, European travel is unusually heavy during July and it is suggested that those contemplating attending the Congress should start making tentative reservations at the earliest possible moment by filling in and returning the card recently mailed to members, to THOMAS COOK & SON's special representative—Harry E. Kornbaum, Transportation Chairman, Rainbow Travel Service, 2817 Classen Boulevard, Oklahoma City 6, Oklahoma, who will correspond directly with each member about his individual travel arrangements to and from the Convention, at the same time keeping our National Officers fully informed of all travel arrangements of its members. Completion of the card CARRIES NO OBLIGATION OR COMMITMENT ON YOUR PART. Send it in today for the complete travel arrangements for our Society.

The general expenses of a meeting of this magnitude are quite large and The Endocrine Society has made a substantial contribution from its treasury for organizational purposes. U.S. pharmaceutical firms have also contributed very generously. However, sufficient funds are not as yet available

to assure the success of this meeting; therefore, *the committee suggests that each of our members make voluntary contributions to this fund.* Please make your checks payable to: The Endocrine Society—First International Congress of Endocrinology. Mark it *Contribution* and mail it to the office of the Secretary-Treasurer, 1200 North Walker Street, Oklahoma City 3, Oklahoma.

Program

The program of the First International Congress of Endocrinology which will be held in Copenhagen, Denmark, July 18–23, 1960, will consist of ten symposia and a round table discussion, along with sessions of communicated papers. The speakers at the symposia and the round table discussion have been invited by the Subcommittee on Program of the Congress. In Symposia #1–8 and in #10, the Subcommittee has also invited a discusser for each principal paper. Symposium #9 will consist of six principal papers and no discussers. The round table discussion will consist of eight sections, each with a principal speaker and three secondary speakers.

(For program see the December 1959 issue of the Journal.)

The American Goiter Association

1960 Annual Meeting and the 4th International Goiter Conference

The next Annual Meeting of the American Goiter Association will be held in *London, England, July 5–9, 1960*, at Church House, Dean's Yard, Westminster, London, S.W.1. This meeting, co-sponsored with the London Thyroid Club, will be the Fourth International Goiter Conference. For further information write Dr. Selwyn Taylor, Royal Society of Medicine, Dering Yard, 67c New Bond Street, London, W.1, England.

John C. McClintock, *Secretary*,
149½ Washington Avenue,
Albany 10, N.Y.

1960 Laurentian Hormone Conference

The 1960 Laurentian Hormone Conference of the AAAS will be held at Mont Tremblant Lodge, Mont Tremblant, Quebec, during the week *September 4–9*. Investigators interested in attending this Conference should make application to the Committee on Arrangements of the Laurentian Hormone Conference, 222 Maple Ave., Shrewsbury, Massachusetts, at an early date and in any event *no later than May 13, 1960*. A conference rate of $14.00 per day per person is extended to all invited participants. Since the number of our participants is necessarily limited by available accommodations, all applications are screened and invitations to attend are issued by the Committee on Arrangements by the second week in June. The following program has been arranged.

PROGRAM

I. Testes Function

Function of normal and abnormal testicular interstitial cells in the mouse.
Robert A. Huseby, Univ. of Colorado, Denver; *Oscar Dominguez and Leo T. Samuels*, Univ. of Utah, Salt Lake City.
Sunday evening, September 4

Genetic aspects of male hypogonadism.
H. Nowakowski and W. Lenz, Univ.-Klinik und Poliklinik, Hamburg-Eppendorf, Germany.
Monday morning, September 5

II. Hormones and Reproduction

Erucic acid and reproduction.
Robert L. Noble, Univ. of Western Ontario, London, Canada.
Monday morning, September 5

Comparative physiology of reproduction and endocrinology of female domestic animals.
A. V. Nalbandov, Univ. of Illinois, Urbana, Illinois.
Monday evening, September 5

Estrogen metabolism in the human fetus and new-born.
E. Diczfalusy, Karolinska Institute, Stockholm, Sweden.
Tuesday morning, September 6

Placental transmission of hormones.
Claude Migeon, Johns Hopkins Hospital, Baltimore; *Jean Bertrand*, Hôpital Edouard Herriot, Lyon, France; and *Carl Gemzell*, Karolinska Institute, Stockholm, Sweden.
Tuesday morning, September 6

The metabolic influence of progesterone in man.
Richard L. Landau, Univ. of Chicago, Chicago, Illinois.
Tuesday evening, September 6

III. Aldosterone

Mechanisms regulating the secretion and metabolism of aldosterone.
James O. Davis, National Heart Institute, Bethesda, Maryland.
Wednesday morning, September 7

The role of aldosterone in experimental nephrosis in the rat.
Claude J. P. Giroud, Montreal Children's Hospital; *Norman Kalant*, Jewish General Hospital, Montreal; *R. H. Despointes and D. Das Gupta*, Montreal Children's Hospital, Montreal.
Wednesday morning, September 7

Clinical endocrinology of aldosterone.
Jerome Conn, University Hospital, Ann Arbor, Michigan.
Thursday morning, September 8

IV. Hormones and Electrolyte Metabolism

Comparative physiology and pharmacology of the neurohypophysis.
W. H. Sawyer, Columbia University, College of Physicians and Surgeons, New York.
Thursday morning, September 8

The effects of neurohypophyseal hormone on permeability and transport in living membrane.
Alexander Leaf and Richard M. Hays, Massachusetts General Hospital, Boston.
Thursday evening, September 8

V. Hormones and Organic Metabolism

The regulation of glucose uptake in muscle by hormones and other factors.
C. R. Park, Vanderbilt University, Nashville, Tennessee.
Friday morning, September 9

The direct action of hormones on liver metabolism.
Leon L. Miller, University of Rochester, Rochester, New York
Friday morning, September 9

Children's Hospital of Philadelphia

Two Short or Refresher Courses will be given in early June 1960, by the Children's Hospital of Philadelphia and the Graduate School of Medicine, University of Pennsylvania:

1) *Pediatric Advances*, May 30–June 3, 1960, conducted by the Staff of The Children's Hospital of Philadelphia. The curriculum will consist of clinics, demonstrations and panel discussions in selected aspects of contemporary pediatrics in which important advances are being made. Tuition: $115.00.

2) *Practical Pediatric Hematology*, June 6–10, 1960, conducted by Irving J. Wolman, M.D., Thomas R. Boggs, Jr., M.D. and other members of the Hematology Department of the Children's Hospital of Philadelphia. Tuition: $125.00.

Inquiries should be addressed to Irving J. Wolman, M.D., Director of Post-Graduate Education, The Children's Hospital of Philadelphia, 1740 Bainbridge Street, Philadelphia 46, Pa.

Book Register

Colloque d' endocrinologie, 1959. Thyroïde et exploration thyroïdienne: Médullo-surrénale Proceedings of the 5th Reunion of Endocrinologists of the French Language, Paris, June 29, July 1, 1959. Published with the assistance of PROF. A. SOULAIRAC. In the section on the thyroid there is a review and discussion of the various procedures for clinical investigation of thyroidal function. Problems regarding the physiopathology of the thyroid are also given a prominent place. These include the phenomena of antithyroid autoimmunization; hyperthyroidism and the metabolism of calcium; and the problem of exophthalmos. In the section on the adrenal medulla, the most recent researches in histophysiology and physiology are presented. The subjects include the regulatory role of adrenaline on the central nervous system and the fundamental homeostatic function of this hormone; there is also a chapter on pheochromocytoma; 420 pages; 129 figures; 1 color plate; 1959. A publication of Annales d'endocrinologie; Masson et Cie, Editeurs, Paris, France.

Communications from the Department of Hygiene, University of Helsinki, 1955–1959. By W. LOJANDER. An outline of the teaching and research work in this Department; 46 pages; 1959. University of Helsinki, Helsinki, Finland.

Der endogene Jodhaushalt des Menschen und seine Störungen. By DOZ. DR. E. KLEIN, Oberarzt der 2. medizinischen Klinik und Poliklinik der medizinischen Akademie Dusseldorf, with a foreword by PROF. DR. K. OBERDISSE, Düsseldorf. Various sections of the book deal with the development of research in the biochemistry of iodine; normal iodine metabolism; application to diagnosis of thyroidal functional states; biochemical methods, and findings in the thyroid, blood and peripheral tissues; euthyroid goiter; hyperthyroidism; hypothyroidism and cretinism; euthyroid endocrine ophthalmopathies; thyroidal abnormalities without hypothyroidism; neoplasms of the thyroid; endogenous iodine metabolism in extrathyroidal diseases; antithyroidal substances; and the influence of exogeneous iodine-containing preparations; extensive bibliography; 171 pages; 35 illustrations; 1960. Georg Thieme Verlag, Stuttgart. In U.S.A. and Canada, Intercontinental Medical Book Corporation, New York 16, N. Y. Price DM24, or $5.70.

Données actuelles sur la physiologie et la pathologie clinique des parathyroïdes. By B. COURVOISIER with the collaboration of E. BERTHOUD, G. ZAHND and A. GRANDJEAN, Policlinique Universitaire de Médecine de Genève, Directeur, PROF. E. MARTIN. The 6 chapters cover various aspects of the metabolism of calcium-phosphorus metabolism and parathyroid function, the action of the parathyroids on the renal excretion of phosphorus and on the physiology and structure of bone, the relations between the different points of action of the parathyroids and the nature of the parathyroid secretion, the regulation of parathyroid function, and the clinical aspects of parathyroid pathology—hyperparathyroidism and hypoparathyroidism; extensive bibliography; 94 pages; 22 figures; 1959. Benno Schwabe & Co., Basel, Switzerland. In U.S.A. and Canada, Intercontinental Medical Book Corporation, New York 16, N. Y. Price $2.25.

Introduction to Colposcopy. A Diagnostic Aid in Benign and Preclinical Cancerous Lesions of the Cervix Uteri. By KARL A. BOLTEN, M.D., Gynecologist and Research

Associate, Obstetric and Gynecologic Pathology, University of Oklahoma School of Medicine, Oklahoma City, Oklahoma; and, cooperating in pathology, WILLIAM E. JAQUES, M.D., Chairman and Professor of Pathology, University of Oklahoma School of Medicine; with Forewords by JOE VINCENT MEIGS, M.D. and DANIEL W. GOLDMAN, M.D. The book is written by a doctor who has had a great deal of experience, both in Europe and this country, with colposcopy—a technic which is of outstanding value in the study of cancer of the cervix. The chapters include a description of the colposcope, colposcopy and cytology, the technic of colposcopy, the findings in benign conditions of the cervix and vagina, atypical colposcopic changes, and the histopathologic aspect, with colposcopic and clinical correlations; 113 pages; 53 illustrations (28 in color); 1960. Grune & Stratton, Inc., New York 16, N. Y. Price $7.75.

Recent Advances in Neuro-physiological Research. Psychiatric Research Report No. 11 of the American Psychiatric Association, edited by D. EWEN CAMERON, M.D., Professor and Chairman, Department of Psychiatry, McGill University and Director, Allan Memorial Institute of Psychiatry, and MILTON GREENBLATT, M.D., Assistant Supt. and Director of Research, Massachusetts Mental Health Center and Associate Clinical Professor of Psychiatry, Harvard Medical School. This volume presents the scientific papers and discussions of a Regional Research Conference held November 8–9, 1957 in Montreal, Canada, under the Joint Auspices of the Department of Psychiatry, McGill University and the American Psychiatric Association's Committee on Research 1957–58. The papers represent new research thinking from several areas, and include articles on communication, memory disturbance, electrophysiology, animal behavior, and the biological basis for psychopathology; 136 pages; 1959. American Psychiatric Association, 1700 Eighteenth Street N.W., Washington 9, D.C. Price $2.00.

Safe Handling of Radioactive Isotopes in Medical Practice. By EDITH H. QUIMBY, Sc.D., Professor of Radiology (Physics), College of Physicians and Surgeons, Columbia University, New York, N. Y. As stated in the Preface, the book is written for technicians, nurses, doctors, or anyone else engaged in isotope work in a hospital or medical practice. The aim has not been to provide a complete manual on radiation safety, but rather to furnish basic information that will give the radiation worker a feeling of assurance that he can meet nonroutine situations if they arise. The chapters deal with the nature of radioactivity, diagnostic and therapeutic uses of radioactive isotopes, instructions for care of the patient following administration of isotopes, precautions in the hospital and the "hot" laboratory, radiation hazards, procedures for coping with possible emergencies, management of radioactive contamination, disposal of radioactive waste, methods for determining exposure rates and maintaining low dose rates, problems of the surgeon in cases of emergency surgery, and problems of medical personnel, the autopsy pathologist and the funeral director in the event of death of the radioactive patient; 130 pages; 1960. The Macmillan Company, New York 11, N. Y. Price $4.50.

Transactions of the Josiah Macy, Jr. Foundation Conferences. Publications Office, 16 West 46th Street, New York 36, N. Y.

Gestation. Fifth Conference, March 11–13, 1958, Princeton, N. J. Edited by CLAUDE A. VILLEE, Ph.D., Assistant Professor of Biological Chemistry, Harvard Medical School, Boston, Massachusetts. Contents—The Biology of the Placenta, Circulation in the Placenta, Venous

Drainage from the Uterus, and Genetic Factors in Prolonged Gestation; 262 pages; 114 figures; 13 tables; 1959. Price $5.75.

Polysaccharides in Biology. Fourth Conference, May 21–23, 1958, Princeton, N. J. Edited by GEORGE F. SPRINGER, M.D., William Pepper Laboratory of Clinical Medicine and Department of Microbiology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania. Contents—Chondroitin Sulfates, The Biology of Chondroitin Sulfates, Heparin and Related Substances, The Mast Cell, Biosynthesis of Sulfated Polysaccharides, Synthetic Nonbiological Sulfated Polysaccharides, and Sulfated Polysaccharides of Plants; 326 pages; 90 figures; 52 tables; 1959. Price $5.95.

The Central Nervous System and Behavior. Second Conference, February 22–25, 1959, Princeton, N. J., in collaboration with The National Science Foundation. Edited by MARY A. B. BRAZIER, Ph.D., Neurophysiological Laboratory, Massachusetts General Hospital, Boston, Massachusetts. Contents—The Limbic System with Respect to Two Basic Life Principles, The Hippocampus and Higher Nervous Activity, Reversible Decortication and Behavior, Electroencephalographic Studies in Conditional Reflex Formation in Man, and Impressions of the Colloquium on Electroencephalography and Higher Nervous Activity, Moscow, USSR, October 1958; 358 pages; 88 figures (2 in color); 1959. Price $4.75.